COMPARATIVE EVALUATION OF ANTIBACTERIAL ACTIVITY AND DENTINAL TUBULE PENETRATION DEPTH OF AH PLUS, REALSEAL SELF-ETCH AND iROOT SP ROOT CANAL SEALERS

- AN IN VITRO STUDY

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CERTIFICATE

This is to certify that **Dr. SHIVANI ,** Post Graduate student (2010 - 2013) in the Department of Conservative Dentistry and Endodontics, has done this dissertation titled "**COMPARATIVE EVALUATION OF ANTIBACTERIAL ACTIVITY AND DENTINAL TUBULE PENETRATION DEPTH OF AH PLUS, REALSEAL SELF -ETCH AND iROOT SP ROOT CANAL SEALERS – AN IN VITRO STUDY"** under my direct guidance and supervision in partial fulfilment of the regulations laid down by **The Tamil Nadu Dr. M.G.R. Medical University, Guindy, Chennai – 32** for **M.D.S.** in Conservative Dentistry and Endodontics (Branch IV) Degree Examination.

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DECLARATION

TITLE OF DISSERTATION	Comparative Evaluation of Antibacterial activity and dentinal tubule penetration depth of AH Plus, RealSeal self-etch, iRoot SP root canal sealers – An in Vitro Study
PLACE OF THE STUDY	Tamil Nadu Government Dental College & Hospital, Chennai – 3.
DURATION OF THE COURSE	3 YEARS
NAME OF THE GUIDE	DR. B. RAMAPRABHA
HEAD OF THE DEPARTMENT	DR. M. KAVITHA

I hereby declare that no part of dissertation will be utilized for gaining financial assistance or any promotion without obtaining prior permission of the Principal, Tamil Nadu Government Dental College & Hospital, Chennai – 3. In addition I declare that no part of this work will be published either in print or in electronic media without the guide who has been actively involved in dissertation. The author has the right to preserve for publish of the work solely with the prior permission of Principal, Tamil Nadu Government Dental College & Hospital, Chennai - 3.

HOD **GUIDE** **Signature of the candidate**

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This agreement herein after the "Agreement" is entered into on this day Dec. 2012 between the Tamil Nadu Government Dental College and Hospital represented by its **Principal** having address at Tamil Nadu Government Dental College and Hospital, Chennai - 600 003, (hereafter referred to as, 'the college')

And

Mrs. Dr. B. Ramaprabha aged 43 years working as **Professor** in Department of Conservtive Dentistry & Endodontics at the college, having residence address at 191/5, Green Fields Apts. R-30A, Ambattur, Thirumangalam High Road, Mugappair, Chennai – 101 (herein after referred to as the 'Principal Investigator')

And

Miss. Dr. Shivani aged 27 years currently studying as **Post Graduate student** in Department of Conservtive Dentistry & Endodontics, Tamilnadu Government Dental College and Hospital, Chennai - 3 (herein after referred to as the 'PG student and co-investigator').

Whereas the PG student as part of his curriculum undertakes to research on "**COMPARATIVE EVALUATION OF ANTIBACTERIAL ACTIVITY AND DENTINAL TUBULE PENETRATION DEPTH OF AH PLUS, REALSEAL SELF-ETCH, iROOT SP ROOT CANAL SEALERS – AN IN VITRO STUDY"** for which purpose the Principal Investigator shall act as principal investigator and the college shall provide the requisite infrastructure based on availability and also provide facility to the PG student as to the extent possible as a Co-investigator.

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10. If any dispute arises as to the matters related or connected to this agreement herein, it shall be referred to arbitration in accordance with the provisions of the Arbitration and Conciliation Act, 1996.

In witness whereof the parties herein above mentioned have on this the day month and year herein above mentioned set their hands to this agreement in the presence of the following two witnesses.

College represented by its **Principal** **PG Student**

Witnesses **Student Guide**

1.

2.

ABSTRACT

AIM

The purpose of this in vitro study was to evaluate and compare the antibacterial activity and dentinal tubule penetration depth of three root canal sealers.

MATERIALS AND METHODS

The sealers to be analyzed were Group I (AH Plus), Group II (RealSeal self-etch) and Group III (iRoot SP). Direct contact test (DCT) was used to assess the antibacterial activity of tested sealers when in contact with Enterococcus faecalis. The materials were examined after 7, 3, 1 day aging samples in phosphate-buffered–saline and freshly mixed. For dentinal tubule penetration depth 30 single rooted human incisiors were selected, divided into three groups (n=10) and filled with single cone technique using tested sealers. The specimens were sectioned longitudinally and prepared for observation using Scanning Electron Microscope.

RESULTS

Group I showed significantly greater antibacterial activity as compared to Group II and III over a period of 7 days time. There was no statistically significant difference in antibacterial activity between Group II and III**.** The maximum mean penetration depth of Group III (108.57µm) was significantly higher than that of Group I (64.89µm) and II (63.53µm). There was no statistically significant difference in penetration depth between Group I and Group II**.**

CONCLUSION

Within limitations of this study, the new ceramic based root canal sealer iRoot SP performed better as compared to existing resin-based sealers.

Keywords: AH Plus, RealSeal self-etch, iRoot SP, Antibacterial activity, Direct contact test, Sealer penetration, Scanning Electron Microscope

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Introduction

Microbes and microbial products are the main etiologic factors of pulpitis and apical periodontitis[28]. Endodontic microflora forms a complex biofilm, which is resistant to antibacterial medicaments or irrigants used in endodontics. Enterococcus faecalis is a gram-positive bacterium often isolated in persistent root canal infections. It is a resilient bacterium frequently recovered from obturated root canals with signs of apical periodontitis. When established in the dentinal tubules, it is difficult to eliminate this species through root canal medication.

The main objectives of root canal treatment are the elimination of microorganisms from the root canal space followed by complete fluid tight seal of canal system to prevent re-infection. Chemomechanical cleaning and shaping, followed by the three – dimensional obturation of the root canal space, are common procedures used to achieve this goal.

However, with thorough chemo-mechanical preparation using various irrigants such as sodium hypochlorite and medicaments it is difficult or even impossible to eliminate completely all organisms from the canal space. All these are consequences of complex root anatomy. Root canal anatomy is the most complex anatomy of human body having fins, isthmi, lateral canals, accessory canals providing a gateway for microbes.

Filling may be able to overcome some of the limitations of chemomechanical preparation. The main aim of filling is firstly to eliminate all avenues of leakage from the oral cavity and periradicular tissues into the root canal system by creating a fluid tight seal. Secondly, to eliminate space and seal within the root canal system any irritants that cannot be fully removed during cleaning and shaping procedures.

Thus, the root canal filling material should prevent coronal re-infection and entomb remaining bacteria within the canal space. Consequently, the use of root canal filling materials with antibacterial activity is considered beneficial in effort to further reduce the number of remaining microorganisms and to eradicate the infection.

The ability of root canal filling materials to penetrate into the dentinal tubules is regarded as a relevant aspect in the prevention of re-infection of dentinal tubules and of the root canal itself. Therefore, it might be advantageous if the sealer exerts some antibacterial activity and penetration depth in dentinal tubules as the last element in the treatment regimen.

Grossman had suggested requirements for a root canal sealer. Over the years, various root canal sealers are used in conjunction with a biologically acceptable semisolid or solid obturating material to establish an adequate seal of root canal system. But none have proved to possess all the ideal characteristics. Therefore, search for an ideal root canal sealer is continuing.

AH Plus (Dentsply) is an epoxy resin-based sealer derived from AH-26, which was introduced in 1954. Various studies have been done on AH Plus sealer and all these studies regarding antibacterial effect of AH Plus have given mixed results[3, 20].

RealSeal dual cure self-etch sealer (Sybron Endo) and Epiphany self-etch (Pentron Clinical Technologies) are resin-based sealers. These resin-based sealers also have adhesion to the radicular dentin and to solid filling materials forming a ‘monoblock’ that has good adaptation. Recently, a new version of RealSeal self-etch sealer, based on

the self-adhesive cement concept was introduced with the promise of optimizing clinical performance with a simplified one-step application procedure.

iRoot SP (Innovative Bioceramix, Vancouver, Canada; also known as EndoSequence BC sealer, Brasseler , Savannah, GA) is a newly introduced root canal sealer based on a calcium silicate composition, which requires the presence of water to set and harden.

Although there are limited investigations available regarding the antimicrobial activity and dentinal tubule penetration depth of Epiphany self-etch (Pentron Clinical Technologies), having composition similar to RealSeal self-etch, but as such there is no study regarding RealSeal self-etch root canal sealer. Due to the difference in manufacturing companies both these sealers may have different physiochemical properties.

There is information available regarding the antimicrobial activity of iRoot SP root canal sealer but no information related to its penetration depth in dentinal tubules.

Therefore, the purpose of this study is to determine the antibacterial activity and dentinal tubule penetration depth of AH Plus, RealSeal self-etch and iRoot SP root canal sealer.

Aim & Objectives

AIM:

To evaluate the antibacterial activity and dentinal tubule penetration depth of AH Plus, RealSeal self-etch and iRoot SP root canal sealers.

OBJECTIVES:

To evaluate the antibacterial property of AH Plus, RealSeal self-etch and iRoot SP sealers which were allowed to set for 7 days, 3 days, 24 hours after mixing and freshly mixed samples using, Direct contact test against the bacteria Enterococcus faecalis.

To evaluate the dentinal tubule penetration depth of AH Plus, RealSeal self-etch and iRoot SP root canal sealers by Scanning Electron Microscope at coronal, middle and apical third of root canal.

Review of Literature

Grossman had listed the ideal requirements of sealers in 1982 and found that sealers should have bactericidal or bacteriostatic property to give a microbial free three dimensional filling in root canal. Gutta-percha is the most common filling material used in root canals. Though it has poor sealing ability and lacks antimicrobial activity, which necessitates the endodontic sealer to be antibacterial.

Jose F. Siqueira, Amauri Favieri et al (2000)[33] investigated and compared the antimicrobial effects and the flow rate of the following sealers: Kerr Pulp Canal Sealer EWT, Grossman's Sealer, ThermaSeal, Sealer 26, AH Plus, and Sealer Plus. All root canal sealers tested showed some antimicrobial activity against most of the microorganisms. There were no significant differences between the materials tested.

Mario Roberto Leonardo, Lea Assed Bezerra da Silva et al (2000)[39] evaluated the antimicrobial activity of four root canal sealers AH Plus, Sealapex, Ketac Endo, and Fill Canal, two calcium hydroxide pastes Calen and Calasept, and a zinc-oxide paste. Seven bacterial strains were used, six of them standard; *Micrococcus luteus* ATCC 9341, *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus epidermidis* ATCC 12228, *Escherichia coli* ATCC 25922, and *Enterococcus faecalis* ATCC 10541. Activity was evaluated using the agar diffusion method. *Enterococcus faecalis* was not inhibited by zinc-oxide and *Pseudomonas aeruginosa* was not inhibited by AH Plus, Fill Canal, and the zinc-oxide-based paste. In conclusion sealers and pastes presented antimicrobial activity in vitro and culture medium optimization with 0.05 g% TTC gel facilitated observation of the inhibition halos.

Chung-Chih Lai, Fu-Mei Huang et al (2001)[7] evaluated the antimicrobial properties of four commonly used endodontic sealers: two epoxy-resin-based sealers AH26, AH Plus, one zinc-oxide eugenol-based sealer (N2), and one calcium hydroxide-based sealer Sealapex. The testing microbes were four facultative anaerobic species Streptococcus mutans, Streptococcus sanguis, Escherichia coli, and Staphylococcus aureus and four obligate anaerobic species Porphyromonas gingivalis, Porphyromonas endodontalis, Fusobacterium nucleatum, and Prevotella intermedia. N2 containing formaldehyde and eugenol proved to be the most effective against the microorganisms.

Gurkan Gur, Semra Sevimay, Aykut Misirligil et al (2002)[22] determined the antimicrobial activity of 8 root canal sealers, using the agar diffusion inhibitory test. The sealers used were Endomethasone, AH26, AH Plus, Sultan, Kerr Pulp Canal Sealer, CRCS Sealapex, and RoekoSeal Automix (RSA). The microorganisms used were Streptococcus mutans, Enterococcus faecalis, Lactobacillus casei, Bacillus subtilis, and Staphylococcus aureus. The freshly mixed sealers were placed into the prepared wells of agar plates inoculated with the test microorganisms. After 24h, 48h, and 72h periods of incubation, the zones of inhibition of bacterial growth were observed and measured. The best antimicrobial activity was with Endomethasone, followed in descending order by AH 26, Sultan, Kerr Pulp Canal Sealer, CRCS, Sealapex, AH Plus. No zone of inhibition was seen with the use of RSA. Concerning the time span, not an important correlation was found with the effect of sealers.

Andre K. Mickel, Tuan H. Nguyen et al (2003)[1] evaluated the antimicrobial activity of four root canal sealers Sealapex, Roth 811, Kerr EWT, and AH-Plus on *E. faecalis*. Seventeen blood-agar plates were inoculated with *E. faecalis* using the Lawn

technique. The zones of inhibition were measured at 24 and 48 h. Roth 811 showed the largest zone of inhibition (1.1 mm), followed by Sealapex (0.8 mm) and Kerr EWT (0.5 mm), whereas AH-Plus had no antimicrobial activity. There was no difference in the zones of inhibition between the 24- and 48-h time periods.

Funda Kont Çobankara et al (2004)[16] evaluated the antibacterial activity of five different root-canal sealers RoekoSeal, Ketac-Endo, AH Plus, Sealapex, Sultan. With the use of *Enterococcus faecalis* as a test organism, both the agar-diffusion test (ADT) and direct-contact test (DCT) were performed. Ketac-Endo, Sultan, and AH Plus had similar results for DCT. These sealers were more potent bacterial-growth inhibitors than Sealapex and RoekoSeal. According to ADT, RoekoSeal showed no antibacterial activity. There was no significant difference among AH Plus, Sealapex, and Sultan. Ketac-Endo demonstrated lower antimicrobial activity than these sealers. Time had no effect on the antibacterial activity of the tested sealers. The antibacterial efficiency of the materials varied according to the tests used. It was concluded that the technique, time, and ingredients of the tested material can affect the results of the microbiological studies.

Brenda Paula Figueiredo de Almeida GOMES et al (2004)[5] analyzed the antimicrobial properties of five endodontic sealers: Endo Fill, Endomethasone, Endomethasone N, Sealer 26 and AH-Plus, against the following microorganisms: *Candida albicans, Staphylococcus aureus, Enterococcus faecalis, Streptococcus sanguis* and *Actinomyces naeslundii.* The sealers were tested immediately, 24 h, 48 h and 7 days after manipulation. The direct contact method through the observation of the microbial growth in liquid medium and the agar diffusion test were used to evaluate the antimicrobial properties of the sealers. The results in both methodologies used, showed

that immediately after manipulation, Endo-Fill and Endomethasone demonstrated the highest antimicrobial activity, with no statistically significant difference between them. Sealer 26 demonstrated the lowest antimicrobial activity. At all other times after manipulation, there were no statistically significant differences among all the sealers tested. In conclusion, none of the sealers totally inhibited the growth of the microorganisms. Furthermore, the antimicrobial activity of each sealer decreased with time and was dependent upon the microbial susceptibility to them.

G. Kayaoglu, H. Erten, T. Alaçam, D. Orstavik et al (2005)[17] investigated the antimicrobial activity of root canal sealers on *Enterococcus faecalis*, either allowing or avoiding direct contact between sealers and bacteria. In the direct contact test, MCS and AH Plus killed the bacteria to a level below the detection limit. They were followed in decreasing order of efficacy by Grossman's sealer, Sealapex and Apexit. In the membrane-restricted contact test, the sealers ranked: MCS, AH Plus, Grossman's sealer, Apexit and Sealapex, in descending order of antibacterial potency. MCS, AH Plus and Grossman's sealer significantly reduced the number of viable bacteria in both tests. Sealapex and Apexit were not statistically different from control. MCS, AH Plus and Grossman's sealer were effective in reducing the number of cultivable cells of *E. faecalis*. Calcium hydroxide-based sealers, Sealapex and Apexit were ineffective in this short-term experiment.

Aravind, V Gopikrishna, D Kandaswamy et al (2006)[3]evaluated the antimicrobial efficacy of a traditional zinc-oxide eugenol based sealer Tubliseal with a iodoform incorporated zinc-oxide eugenol based sealer Endoflas FS, a calcium hydroxide based sealer Apexit and the epoxy resin based sealers AH PLUS and RC Seal, against the

microorganisms Enterococcus faecalis and Candida albicans. The method employed to test the antimicrobial efficacy was the Kirby-Bauer method (Agar Disc Diffusion). The antimicrobial efficacy of an iodoform incorporated zinc-oxide eugenol based sealer, Endoflas FS against Enterococcus faecalis and Candida albicans was statistically superior to the rest of the test groups. Endoflas FS performed far better than even the controls being employed (Amoxycillin and Nystatin) respectively. Tubliseal, a zinc-oxide eugenol based sealer also showed significant antimicrobial properties, but was statistically inferior to Endoflas FS Apexit, a calcium hydroxide based sealer did not show significant antimicrobial efficacy against both Enterococcus faecalis and Candida albicans. AH PLUS and RC seal, epoxy resin based sealers showed no antimicrobial properties whatsoever.

Daniela Cristina Miyagak, Elaine Manso Oliveira Franco de Carvalho (2006)[9] evaluated the antimicrobial activity of the endodontic sealers: N-Rickert, Sealapex, AH Plus, Mineral Trioxide Aggregate (MTA) and portland cement. The Agar diffusion method was used in plates previously inoculated with the following microorganisms: *C. albicans, S. aureus, E. faecalis, E. coli*. The diameters of microbial inhibition zones were measured after 24 hours of incubation in kiln at 37°C. It concluded that sealers AH Plus and N-Rickert presented antimicrobial activity against *C. albicans*, *S. aureus,* and *E. coli*. No antimicrobial activity in MTA, Sealapex and portland cement was observed. N-Rickert presented the largest inhibition zones varying from 8 to 18 mm, and the microorganism *E. faecalis* was resistant against all sealers tested.

Giuseppe Pizzo, Giovanni M. Giammanco et al (2006)[20] evaluated the antibacterial activity of four endodontic sealers: one epoxy resin sealer AH Plus, two zinc-oxide eugenol (ZOE)-based sealers Endomethasone, Pulp Canal Sealer, and one sealer containing both ZOE and orthophenilphenol Vcanalare. All freshly mixed sealers showed complete inhibition of bacterial growth. Similar results were obtained with the 24-h-old samples, with the exception of AH Plus. Vcanalare was the only sealer still inhibiting bacterial growth 7 days after mixing. In conclusion, the antimicrobial activity of the tested sealers depends on the time interval between mixing and testing. All sealers exhibit bactericidal effect when freshly mixed, but only Vcanalare extended this effect until 7 days after setting.

Eldeniz AU, Orstavik D et al (2007)[13] investigated the antimicrobial activity against Enterococcus faecalis of new root canal sealers, Epiphany used with Resilon, EndoREZ, RC Seale, Acroseal, GuttaFlow in comparison with established sealers AH Plus, Apexit and RoekoSeal, used with conventional gutta-percha. In the direct contact test, the materials displayed antibacterial activity in the following, decreasing order: RC Sealer, AH Plus, Epiphany, Acroseal, EndoREZ, Hygenic gutta-percha, Activ Point, GuttaFlow, RoekoSeal, Resilon, Apexit. RC Sealer was significantly more active than Epiphany and the materials with less average activity. In the membrane-restricted test, the materials displayed antibacterial activity in the following, decreasing order: RC Sealer, AH Plus, RoekoSeal, Hygenic gutta-percha, Resilon, Activ Point, EndoREZ, GuttaFlow, Epiphany, Acroseal, and Apexit. RC Sealer was significantly more potent than AH Plus and the materials with less average activity. RC Sealer and AH Plus sealers were most

and Apexit was least effective in reducing E. faecalis. Activity of Epiphany was reduced when the E. faecalis cells were separated by a membrane.

Yasuda Y, Kamaguchi A, Saito T et al (2008)[64] compared the antimicrobial activities of a new resin-based SuperBond (SB) Sealer and five other sealers/cements against endodontic pathogens. The antimicrobial activities of SB Sealer, Sealapex, AH Plus, Roeko Seal Automix, Canals N, and Pro Root mineral trioxide aggregate (MTA) were examined using a double-layered method. The microorganisms Staphylococcus aureus, Enterococcus faecalis, Candida albicans, Streptococcus mutans, and Streptococcus sanguinis were used. AH Plus exhibited the highest antimicrobial activity. Pro Root MTA showed no antimicrobial activity against any of the microorganisms tested. SB Sealer offered no antimicrobial advantage over the other sealers tested except for Pro Root MTA.

Neelakantan P, Subbarao CV et al (2008)[44]evaluated the duration of antimicrobial activity of ten root canal sealers Apexit plus, Tubli Seal Xpress, Endoflas FS, Endomethasone, Endomethasone N, AH Plus, Epiphany, EndoRez, Ketac Endo, Roeko Seal against Enterococcus faecalis and Candida albicans by the agar diffusion test. The zones of inhibition were examined immediately and after 24, 48, 72 hours, 5 and 7 days. Against Enterococcus faecalis, Endoflas FS showed the largest inhibitory zones immediately and 24 hours after manipulation whereas, there was no significant difference between Endoflas FS and Endomethasone after 48 hours. Against Candida albicans, Endoflas FS performed better than the other sealers. All the sealers (except AH Plus, Epiphany and Roeko Seal) demonstrated higher antimicrobial action in the first 24 hours

after manipulation. The antimicrobial action of all the sealers (except AH Plus and Roeko Seal which showed no antimicrobial activity in any studied time and Epiphany which ceased to show any antimicrobial action after 24 hours) decreased significantly with time.

Iris Slutzky-Goldberg, Hagay Slutzky et al (2008)[31] evaluated the antimicrobial effects of root canal sealers. The direct contact test (DCT) was used to assess the antibacterial properties of AH Plus, Apexit Plus, Epiphany SE, and RoekoSeal when in contact with Enterococcus faecalis. The materials were examined immediately after setting and 1, 2, 7, and 14 days after aging in phosphate-buffered saline. Apexit Plus had a short-term antibacterial effect of 1 day on E.faecalis, whereas Epiphany SE enhanced bacterial growth for at least 7 days. AH Plus and RoekoSeal were ineffective.

Smadi L, Khraisat A et al (2008)[59] analyzed the antimicrobial activity of Nine root canal sealers 4 resin-based sealers, 3 zinc-oxide eugenol based (ZOE) sealers, and 2 calcium hydroxide based root canal sealers. Three microbial strains Staphylococcus aureus, Candida albicans and Enterococcus faecalis were used in this study. The antimicrobial activity of root canal sealers was tested by using the direct contact test at three time intervals. Staphylococcus aureus: All sealers showed significant differences when freshly mixed except Endorez and sealapex. Candida albicans: Only the 48 hours and the one week preparations of Sealapex showed significant differences. The 48 hrs preparations of Topseal and AH Plus showed significant differences. The ZOE based sealers showed significant differences at all time intervals. Candida albicans: All sealers showed significant differences when freshly mixed except the two calcium hydroxide based sealers that showed no significant differences at all time intervals. Enterococcus

faecalis: Topseal, AH Plus, AH 26, Sealite regular and Acroseal showed significant differences only when freshly mixed. The 48 hours and the week preparations of all root canal sealers showed no significant differences.

Hui Zhang, Ya Shen et al (2009)[28] evaluated the antibacterial effectiveness of 7 different endodontic sealers, AH Plus, Apexit Plus, iRoot SP, Tubli Seal, Sealapex, Epiphany SE, and EndoRez against *Enterococcus faecalis* in vitro. Fresh iRoot SP killed all bacteria in 2 minutes, AH Plus in 5 minutes, EndoRez in 20 minutes, and Sealapex and Epiphany in 60 minutes. Freshly mixed Apexit Plus and Tubli Seal failed to kill all bacteria at 60 minutes. For 1-day and 3-day samples, iRoot SP and EndoRez had the strongest antibacterial activity, followed by Sealapex and Epiphany. Tubli Seal and AH Plus did not show any significant antibacterial activity. Of all the samples, Apexit Plus had the lowest antimicrobial activity. The pH of the sealers could not alone explain their antibacterial effect. In conclusion fresh iRoot SP, AH Plus, and EndoRez killed *E. faecalis* effectively. iRoot SP and EndoRez continued to be effective for 3 and 7 days after mixing. Sealapex and EndoRez were the only ones with antimicrobial activity even at 7 days after mixing.

Claudio Poggio, Marco Lombardini et al (2011)[8] performed an in vitro evaluation of the antibacterial properties of 6 endodontic sealers Endomethasone C, Argoseal, Bioseal Normal, Acroseal, AH Plus, Sicura Seal. The agar diffusion test (well and paper disc methods) with Enterococcus faecalis, Staphylococcus aureus and Streptococcus mutans was used. Diameters of halos formed around the sealers were measured after 24 h and 48 h. Endomethasone C, Argoseal and Bioseal showed the

largest inhibition halos for all the tested microorganisms, while Sicura Seal and AH Plus showed low antibacterial effects. Moreover, the comparison of well method and paper disc methods showed significant statistical differences for all sealers and indicated a dose-dependent antimicrobial effect.

Ines Willershausen, **Angelika Callaway et al (2011)**[29] investigated *in vitro* the cytotoxicity and antibacterial properties of four different endodontic sealers using human periodontal ligament fibroblast cell proliferation and visual analysis of growth inhibition. A silicone (GuttaFlow), silicate (EndoSequence BC), zinc-oxide eugenol (Pulp Canal Sealer EWT) and epoxy resin (AH Plus Jet) based sealer were incubated with PDL fibroblasts (10^4 cells/ml, n = 6) up to 96 h. Cell growth and morphology was visualized by means of fluorescent dyes. Possible antibacterial properties of the different sealers were visualized by means of SEM (*Enterococcus faecalis; Parvimonas micra*). After 72 and 96 h GuttaFlow and EndoSequence BC showed relatively non-cytotoxic reactions, while Pulp Canal Sealer EWT and AH Plus Jet caused a significant decrease of cell proliferation. No antibacterial effect of EndoSequence BC to *P. micra* was found, whereas GuttaFlow showed a weak, Pulp Canal Sealer EWT and AH Plus Jet extensive growth inhibition. Also, no antibacterial effect of GuttaFlow, EndoSequence BC or AH Plus Jet to *E. faecalis* could be detected.

Nawal RR, Parande M et al (2011)[43] tested the antimicrobial efficacy and flow properties of Guttaflow, Epiphany sealer and AH-Plus sealer. With the use of *Enterococcus faecalis ATCC 29212* as a test organism, both the agar diffusion test (ADT) and direct contact test (DCT) were performed. For both the ADT and DCT tests,

Epiphany and AH-Plus sealer reduced the bacterial counts significantly. Epiphany produced a greater reduction in bacterial counts when compared to AH-Plus in both the tests. Guttaflow paste failed to show any antibacterial activity in both ADT & DCT. According to the flow test, all root canal sealers flowed. Epiphany sealer had the maximum flow under the given conditions, followed by AH-Plus sealer and Guttaflow paste.

Sahar Shakouie, Mahsa Eskandarinezhad et al (2012)[56] compared the antimicrobial effect of three different sealers AH-Plus, Adseal and Endofill on *Enterococcus faecalis.* The antimicrobial effect of three sealers was tested by the agar diffusion method. The freshly mixed sealers were placed in prepared wells of agar plates inoculated with *E. faecalis*. The diameter of zone of microbial growth inhibition produced around the wells was measured (in mm) after 3, 5 and 7 days. In all determined intervals, the antibacterial activity of Endofill was significantly higher than other test materials. AH-Plus had moderate effect on *E. faecalis*, while Adseal showed the lowest antibacterial activity on tested bacteria. The Endoffill sealer showed the highest antimicrobial effect compared to AH-Plus and Adseal sealers. Furthermore, the antimicrobial activity of all sealers decreased with time.

SEALER PENETRATION DEPTH IN TUBULES

de Deus G, Gurgel Filho ED et al (2002)[10] studied to evaluate the capacity of penetration of four endodontic sealers EndoFill, Sealapex, AH Plus and Pulp Canal Sealer into dentinal tubules. After filling, the roots were grooved, longitudinally split and examined under a scanning electron microscope (SEM). The focus of observation was the

interface between the dentin and the sealing material. The Rickert sealer (Pulp Canal) presented the maximum penetration depths into the dentinal tubules, and Sealapex, the minimum. The removal of smear layer allowed significant penetration of the sealers.

Karadag LS, Bala O et al (2004)[36] assess the *in vitro* apical microleakage of a resin-based sealer used with two different adhesives. Specimens in Group 1 were filled with gutta-percha, AH Plus sealer, and water-based adhesive system (Syntac Single Component). Group 2 specimens were filled with gutta-percha, AH Plus sealer and acetone-based dentin adhesive (Prime & Bond NT). Specimens of Group 3 were filled with only gutta-percha and AH Plus sealer (no adhesive was applied). Dentin tubule penetration was observed under scanning electron microscopy (SEM). Results showed no statistically significant difference between the materials used.

Andreas B. Kokkas, Asterios Ch. Boutsioukis et al (2004)[2] studied the effect of the smear layer on the penetration depth of three different root canal sealers into the dentinal tubules. After chemomechanical preparation, the samples were randomly divided in two equal groups. The smear layer remained intact in Group A, whereas complete removal of the smear layer was performed in Group B. Ten roots from each group were obturated with laterally condensed gutta-percha points and sealers AH Plus, Apexit, and Roth 811, respectively. Examination in scanning electron microscope revealed that the smear layer obstructed all the sealers from penetrating dentinal tubules. In contrast, smear layer removal allowed the penetration of all sealers to occur to a varying depth.

Gustavo De-Deus, Eduardo Diogo Gurgel-Filho et al (2004)[23] studied to compare the depth of tubular dentinal penetration of sealer in three filling techniques.

Seventy two teeth maxillary central incisors were instrumented and randomly divided in three Groups A, B and C and obturated as following: A: lateral condensation; B: single cone technique and C: warm vertical compaction of gutta-percha. Each sample was sectioned longitudinally and prepared for SEM analysis. There were no significant differences between G1 and G2. The samples filled by warm vertical compaction of gutta-percha presented significantly deeper tubular sealer penetration than lateral condensation and single cone techniques.

Grga D., **Miletic Vesna, Jelic M. et al (2007)**[21] measured layer thickness of 5 endodontic sealers and evaluate sealer distribution and adaptation of Thermafil and sealer within root canals. Root canals were obturated with Thermafil and 1 of 5 different endodontic sealers: AH Plus, Tubliseal, Acroseal, Apexit and Sealapex. Roots were cross-sectioned in 3 levels resulting in 4 sections for scanning electron microscopy (SEM). The layer thickness in decreasing order was: Acroseal > AH Plus > Sealapex > Apexit > Tubliseal. Microgaps between dentinal wall and the obturating material and gutta-percha / carrier could contribute to inadequate adhesion within the root canal and increased microleakage of Thermafil compared to other obturation techniques.

Saman R. Gharib, Patricia A. Tordik et al (2007)[19] assessed the sealer-dentin interface and compared the percentage and average depth of dentinal tubule sealer penetration in the coronal, middle and apical thirds of teeth obturated with the Epiphany Obturation System using 10x and 40x confocal laser scanning microscopy. The Kruskal-Wallis test and post-hoc tests found significantly lower average depth of sealer penetration in apical sections than middle or coronal sections.

E. Balguerie, M. Georgelin-Gurgel et al (2007)[11] studied to evaluate the penetration of 5 root canal sealers into dentinal tubules: a zinc-oxide eugenol based sealer Endobtur, a glass-ionomer sealer Ketac Endo, a calcium-hydroxide based sealer Acroseal, an epoxy-resin-based sealer AH Plus, and a silicon-based sealer Roeko Seal Automix. Ketac Endo was the only sealer which did not penetrate into the dentinal tubules. In the apical third, only AH Plus penetrated the dentinal tubules. In the middle and coronal third, Acroseal and AH Plus showed the best results, ahead of Endobtur and RSA.

K Mamootil, H Messer et al (2007)[34] compared the depth and consistency of penetration of three different root canal sealer cements into dentinal tubules in extracted teeth and to measure the penetration of an epoxy resin-based sealer cement in vivo. Root canals of 50 extracted human pre-molar teeth were prepared and obturated using three different sealer cements based on epoxy resin AH26, zinc-oxide eugenol Pulp Canal Sealer EWT and methacrylate resin EndoREZ. Five teeth filled without sealer were used as controls. Teeth were sectioned and prepared for observation using scanning electron microscopy. A further 12 teeth with a history of successful root filling and subsequent extraction were collected and sectioned. The depth of sealer penetration into dentinal tubules was measured and the consistency and appearance of the sealer within the tubules observed. AH26 demonstrated the deepest penetration (1337 µm), followed by EndoREZ (863 µm) and Pulp Canal Sealer EWT (71 µm). The resin-based sealers appeared to penetrate tubules more consistently. In the clinical cases, all teeth demonstrated sealer penetration to varying depths (98-1490 µm). The depth and consistency of dentinal tubule penetration of sealer cements appears to be influenced by the chemical and physical

characteristics of the materials. Resin-based sealers displayed deeper and more consistent penetration. Penetration depths observed for the epoxy resin-based sealer in vivo were consistent with that found in the experimental model.

Ronald Ordinola-Zapata, Clovis M. Bramante et al (2009)[51] studied to evaluate the percentage of sealer penetration in root canals filled with the Thermafil or RealSeal-1 systems analyzed by confocal laser scanning microscopy (CLSM). Horizontal sections were made at the 3 and 5 mm levels from the apex, and the percentage of sealer penetration in the root canal walls was analyzed using CLSM. Thin layers of sealer (2-30 μm) and sealer tags into dentinal tubules were found in the root canal walls in a high percentage using both techniques at both evaluated levels, with no statistical differences between the techniques

Moon YM, Shon WJ et al (2010)[41] studied to evaluate the effect of different final irrigation regimens on the sealer penetration into dentinal tubules of curved root canals. The samples were divided into 3 groups according to the final irrigation used: Group N (control), 3.5% NaOCl; Group E, 17% ethylenediaminetetraacetic acid (EDTA); and Group EN, 17% EDTA followed by 3.5% NaOCl. All teeth were obturated with gutta-percha and AH Plus sealer labeled with fluorescent dye. Transverse sections at 2 mm (apical) and 5 mm (coronal) from root apex were examined by using confocal laser scanning microscopy. Then, total percentage and maximum depth of sealer penetration were measured. The apical sections in each group showed significantly lower percentage and maximum depth of sealer penetration than the coronal sections. In apical levels, Group E and EN resulted in a higher percentage of sealer penetration than the control

group, but there was no significant difference of maximum depth between Group E and the Control group.

Eric Balguerie, Lucas van der Sluis et al (2011)[15] studied to assess, in vitro, the tubular adaptation and penetration depth and the adaptation to the root canal walls in the apical, middle, and coronal third of the root canal of 5 different sealers used in combination with softened gutta-percha cones. Thereafter, the roots were cross-sectioned and prepared for scanning electron microscopic evaluation. Adaptation of the sealer to the root canal and tubular walls and tubular penetration were assessed. AH Plus, an epoxy resin sealer, showed the best tubular adaptation and penetration. The tubular penetration and adaptation varies with the different physical and chemical properties of the sealers used. AH Plus showed the most optimal tubular penetration and adaptation to the root canal wall of the sealers tested

PG Punitha, K Shashikala et al (2011)[48] evaluated and compared the adaptation of resin based sealers Epiphany, AH Plus and AH 26 to the root canal dentin using scanning electron microscope (SEM). The samples in Group 1 were obturated with AH Plus sealer+ gutta-percha cones, Group 2 with AH 26 sealer + gutta-percha cones and in Group 3 Epiphany sealer + Resilon cones were used as obturating material. The access cavities were restored with composite resin. The sectioned teeth were analyzed under SEM and sealer adaptation to the dentinal walls at apical and middle third was examined. From photomicrographs, epiphany sealer showed better adaptation to root canal dentin followed by AH Plus and AH 26 sealer.

Shokouhinejad N, Sabeti M et al (2011)[58] measured the average depth of dentinal tubule sealer penetration in the middle third of teeth obturated with gutta-percha/AH Plus, Resilon/Epiphany, and Resilon/Epiphany self-etch (SE) using scanning electron microscopy (SEM). The mean values for the average depth of sealer penetration in the middle third of the roots were 22.07 +/- 6.92 µm, 31.56 +/- 6.80 µm, and 21.50 +/- 9.25 µm for AH Plus, Epiphany, and Epiphany SE, respectively. The average penetration depth of Epiphany was significantly higher than that of Epiphany SE and AH Plus. There was no significant difference between the penetration depth of Epiphany SE and AH Plus. It could be concluded that the average penetration for Epiphany into dentinal tubules within the middle third of the roots was significantly deeper than that of Epiphany SE and AH Plus.

S Anil Kumar, Vasundhara Shivanna et al (2011)[54] evaluated the apical sealing ability and adaptation of three resin-based sealers to the dentine. The teeth were prepared and obturated with gutta-percha by a lateral condensation using AH Plus, Endorez and Epiphany sealers. The Epiphany sealer has a better apical sealing ability and adaptation to dentine than the AH Plus and Endorez sealers.

Ravindranath M, Neelakantan P et al (2011)[49] studied to determine sealer penetration into dentinal tubules and sealer thickness with different obturation materials and techniques. Samples were obturated using the lateral condensation technique with either gutta-percha (Group 1) or Resilon (Group 2), using AH Plus (subgroup A) or Epiphany (subgroup B) as a sealer. Other samples were obturated with One-Step Obturator (Group 3) using AH Plus or Epiphany sealer. The sealer thickness and sealer penetration into dentinal tubules was evaluated using stereomicroscopy and analysis of

digital images using AutoCAD software at 5.0 mm, 3.0 mm, and 1.0 mm from the apex. The mean value of sealer thickness for Group 3 was significantly lower than the mean values of the other groups. There was no significant difference in the mean values between subgroups A and B for Group 1 or Group 3, whereas for Group 2, the mean value in subgroup A was significantly higher than the mean value in subgroup B. The greatest average frequency of the penetration of sealer cement was found at the 5.0 mm level, followed by the 3.0 mm level, which, in turn, was greater than at the 1.0 mm level. The thickness of the sealer cement is dependent on the obturation technique employed, while the penetration of the sealer into the dentinal tubules is independent of the obturation technique.

Aysun Kara Tuncer, Safa Tuncer et al (2012)[4] studied to evaluate the effects of different solutions used for final irrigation on sealer penetration into dentinal tubules. The samples were divided into 4 groups according to the final irrigation solution used: (1) the EDTA group: 17% EDTA + 2.5% NaOCl, (2) the maleic acid (MA) group: 7% MA + 2.5% NaOCl, (3) the citric acid (CA) group: 10% CA + 2.5% NaOCl, and (4) the control group: 2.5% NaOCl. All teeth were obturated using the cold lateral condensation technique with gutta-percha and AH 26 sealer labeled with fluorescent dye. Total percentage and maximum depth of sealer penetration were measured using confocal laser scanning microscope. The coronal sections in each group showed a significantly higher percentage and maximum depth of sealer penetration than did the apical and middle sections. In conclusion final irrigation with EDTA, MA, and CA after the use of NaOCl affect the sealer penetration. However, there was no significant difference between these experimental groups (EDTA, MA and CA) in all sections.

Chadha R, Taneja S, et al (2012)[53] evaluated the depth of penetration of three resin-based root canal sealers into the dentinal tubules at the cervical, middle and apical third of the root canal. Root canals of teeth were prepared and obturated using EndoREZ + resin-coated gutta-percha points (Group A), Epiphany + Resilon points (Group B), or AH Plus + gutta-percha (Group C). The teeth were split longitudinally in bucco-lingual direction and viewed under scanning electron microscope. Photographs were taken at cervical, middle and apical levels for all samples and corresponding measurements were made. The results showed that the greatest penetration into dentinal tubules was by EndoREZ sealer (525.2 μ, 327.802 μ and 198.36 μ at cervical, middle and apical third), followed by Epiphany sealer (479.7 μ, 297.212 μ, and 182.22 μ), and the least penetration was seen with AH Plus sealer (224.2 μ, 65.419 μ, and 40.7 μ). In conclusion the penetration depth of EndoREZ and Epiphany into the dentinal tubules is significantly greater than that of AH Plus.

Saurabh S. Chandra, Padmanabhan Shankar et al (2012)[57] studied to evaluate the depth of penetration of 4 different endodontic resin sealers into the radicular dentinal tubules with the aid of confocal microscopy. The samples were obturated with AH Plus, RealSeal, EndoRez, and RoekoSeal resin sealers, respectively. The core material in all the groups was Resilon. The teeth were sectioned at the coronal, middle, and apical thirds and viewed under confocal microscope to determine the depth of penetration of the sealer into the dentinal tubules. The results showed that the maximum penetration was exhibited by RealSeal resin sealer, followed by AH Plus, RoekoSeal, and EndoRez. The coronal third showed the maximum penetration, followed by middle third and least at the apical third. In conclusion RealSeal resin sealer exhibited the maximum penetration.

Chandra Vijay Singh, S Anitha Rao, V Chandrashekar et al (2012)[6] examined in vitro penetration depth of two resin-based sealers AH Plus and Resino Seal and Zinc-Oxide eugenol sealer into the dentinal tubules after removing smear layer by passive ultrasonic irrigation. The results showed that AH Plus had maximum penetration depth into dentinal tubules.

Materials & Methods

MATERIALS USED

GROUP I – AH PLUS SEALER (Dentsply) (Figure 1)

It is an epoxy resin-based sealer supplied as paste-paste system.

Composition

AH Plus paste A

(Epoxide Paste)

- ✓ Bisphenol –A epoxy resin
- ✓ Bisphenol – F epoxy resin
- ✓ Calcium tungstate
- ✓ Zirconium oxide
- ✓ Silica
- ✓ Iron oxide pigments

AH Plus paste B

(Amine Paste)

- ✓ Dibenzyldiamine
- ✓ Aminoadamantane
- ✓ Tricyclodecanediamine
- ✓ Calcium tungstate
- ✓ Zirconium oxide
- ✓ Silica
- ✓ Silicone oil

Group II – RealSeal self-etch sealer (Sybron Endo) (Figure 2)

RealSeal SE sealer incorporates the use of self-etching primers. It is a "dual-cure sealer". RealSeal self-etch (SE) leads to the elimination of separate etching/bonding step. The acidic resin monomers in the self-etch primer are incorporated in RealSeal SE sealer, thus making the technique an all-in-one step. RealSeal SE uses a polymerizable methacrylate carboxylic acid anhydrite (4-META) as the acidic resin monomer, which etches through the smear layer into the underlying radicular dentin.

Composition:

- urethane dimethacrylate (UDMA)
- poly dimethacrylate (PEGDMA)
- ethoxylatedbisphenol A dimethacrylate (EBPADMA)
- bisphenol A glycidyl, methacrylate (BIS-GMA)
- barium borosilicate
- barium sulfate
- bismuth oxychloride
- calcium hydroxide
- photo initiators
- thinning resin

Self-etch primer

- sulfonic acid terminated functional monomer
- HEMA
- Water
- polymerization initiator

Group III – iRoot SP sealer (Innovative Bioceramix, Vancouver, Canada) (Figure 3)

It is a premixed calcium silicate based sealer.

Composition:

- Zirconium oxide
- Calcium silicates
- Calcium phosphate monobasic
- Calcium hydroxide
- Filler
- Thickening agents

Figure1. AH Plus root canal sealer

Figure2. RealSeal self-etch root canal sealer

Figure3. iRoot SP root canal sealer

MATERIALS AND METHODS

The following armamentaria and materials were used in this study.

For preparation of material specimen

- Mixing pad
- Mixing spatula
- Plastic filling instrument

For direct contact test

- 96 well microtitre plate
- Phosphate buffered saline
- Micropipette
- Enterococcus faecalis culture (10^6 bacteria)
- Brain Heart Infusion broth
- Incubator (Guna Enterprises)
- Microplate reader (Epoch Microplate Spectrophotometer, Biotek)

ANTIBACTERIAL PROPERTY

Direct contact test (DCT) by Weiss (1996)[13] was used to study the antibacterial property of the materials tested. Enterococcus faecalis was used as the test organism.

Each Group was tested for

a) Set sealer aged for 7 days in Phosphate buffered saline in humid atmosphere at 37^0 C – sub group a.

b) Set sealer aged for 3 days in Phosphate buffered saline in humid atmosphere at 37^0 C – sub group b.

c) Sealer set for 24 hours in humid atmosphere at 37^0 C – sub group c.

d) Immediately after setting (fresh sample) – sub group d.

PROCEDURE

Sealer placement and aging

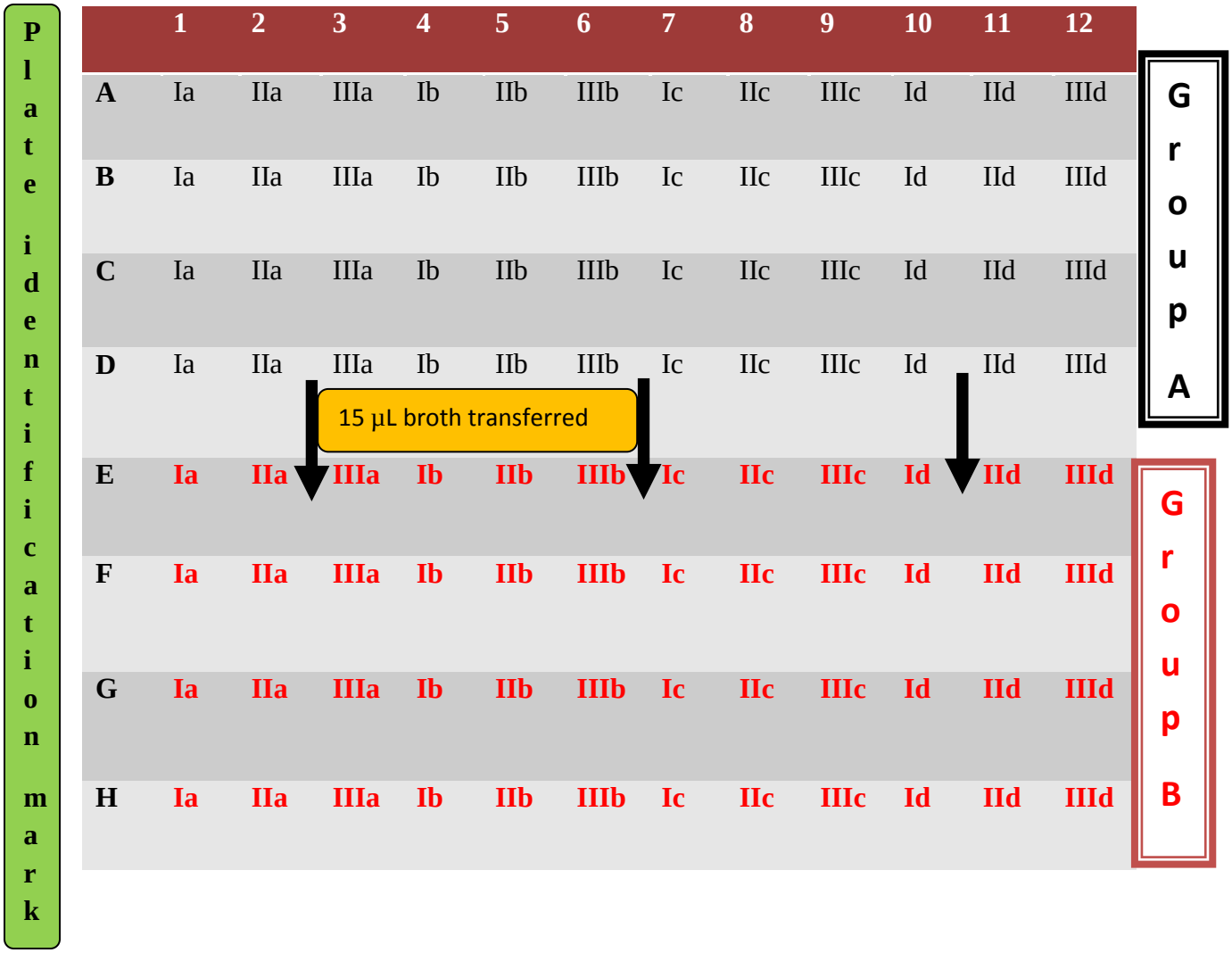
96-well flat bottom microtitre plates were used. Plates were held vertically, that is the plate's surface was maintained perpendicular to the floor plane and the side wall of 4 wells (A1 – D1) were coated with test material from Group I, Group II (A2 – D2), Group III (A3 – D3) and aged for 7 days in phosphate buffered saline in humid atmosphere at 37^0 C – subgroup a. (schematic diagram 1) (Figure 4).

Similarly next 4 wells were coated with test material from Groups I, II, III and aged for 3 days in phosphate buffered saline in humid atmosphere at 37^0 C – subgroup b.

Next set of 4 wells were coated with test material from Groups I, II, III and allowed to set for 24 hours in humid atmosphere at 37^0 C – subgroup c.

Freshly mixed material from each Group I, II, III were coated in another set of 4 wells and allowed to set – designated as fresh sample – subgroup d.

Even and thin coating was achieved by using a small size round ended dental plastic filling instrument. Special care was taken to avoid the material's flow to the bottom of the well, which would interfere with the light path through the microplate well and results in false readings.



Schematic Diagram 1. 96 well microtitre plate showing distribution of test materials.

Group A- in presence of tested material, Group B- in absence of tested material, a- 7 days aged samples, b- 3 days aged samples, c- 24 hrs. aged samples, d- freshly prepared samples

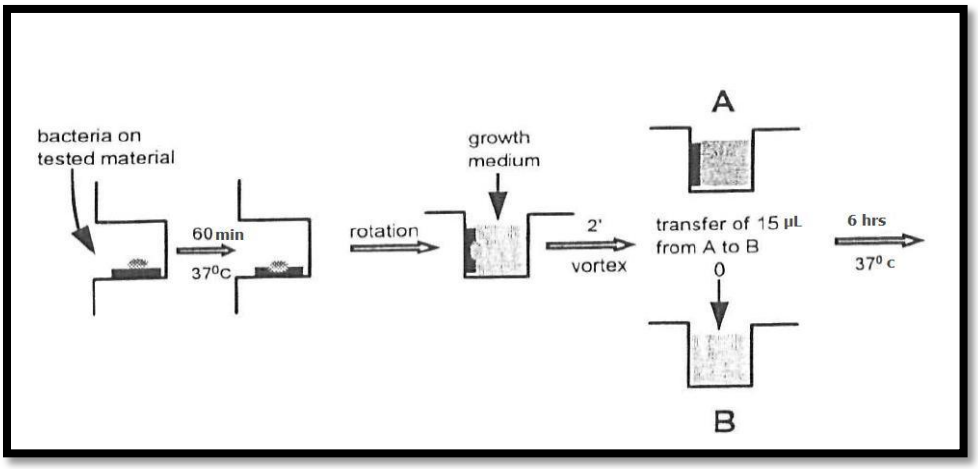
This resulted in set of 4 wells for each subgroup in every group to be tested and designated as Group A wells where growth is monitored in the presence of material.

Preparation of bacterial specimen

Bacteria from frozen stock cultures were grown aerobically to late logarithmic or early stationary phase in brain heart infusion (BHI) broth at 37^0 C. Cells were harvested by centrifugation and re-suspended in fresh medium. Inoculates were prepared by adjusting the cell suspension to predetermined optical densities (OD) corresponding to 10^6 CFU/ml. (Figure 5)

Direct contact test

A 10 µL bacterial suspension (10^6 bacteria) was placed on the test materials. (Figure 6) While the plate remained in vertical position, wells were inspected for evaporation of the suspension's liquid, which occurred within 1 h at 37^0 C. This ensured direct contact between the bacteria and tested material. BHI broth (245 µL) was added (Figure 7) to each of the Group A wells and gently mixed for 2 min. 15 µL were then transferred from Group A wells respectively to an adjacent set of 4 wells containing fresh (215 µL) designated as Group B. (arrows in schematic diagram 1,2) (Figure 8)



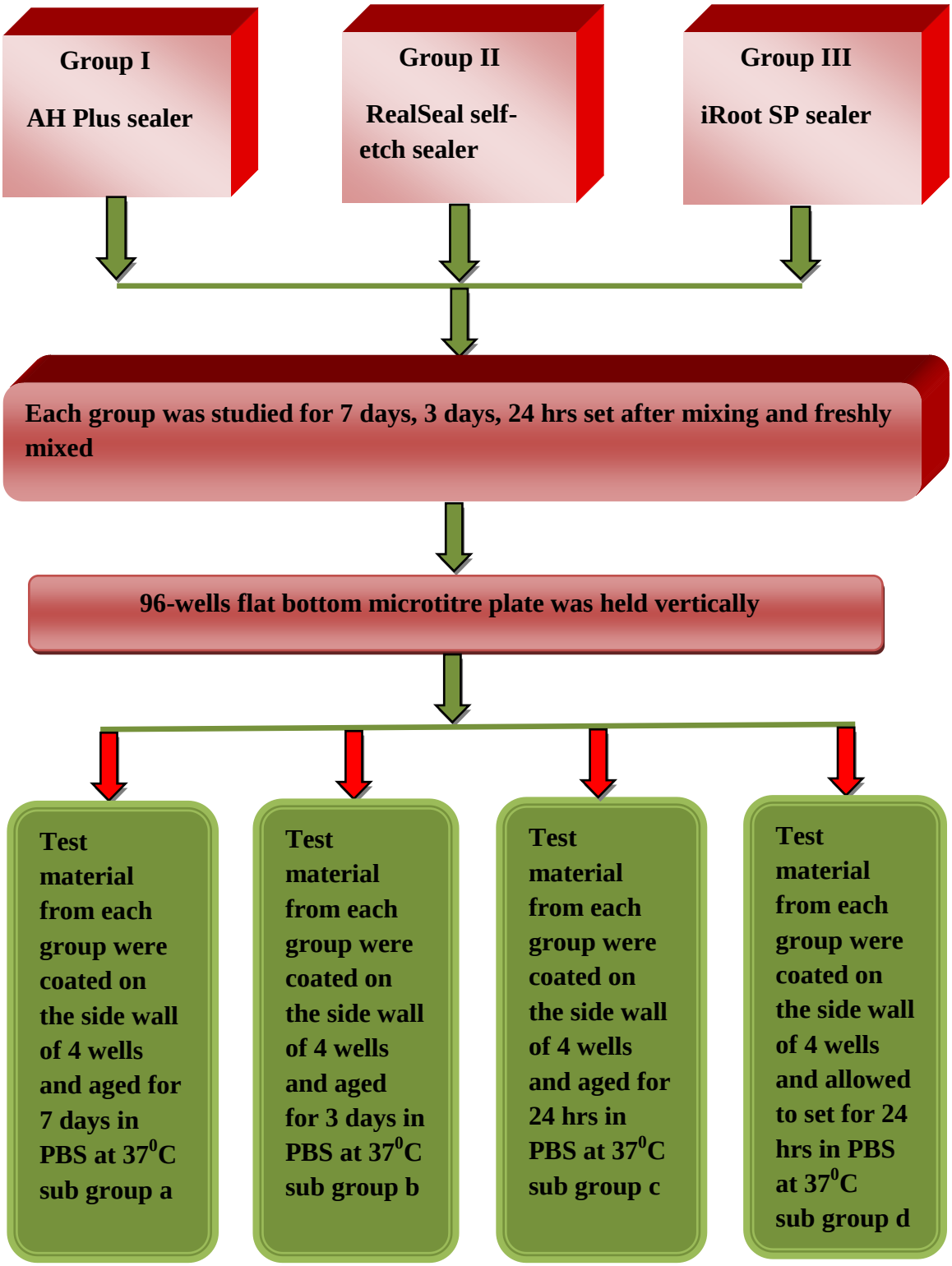
Schematic Diagram 2. Procedure for direct contact test

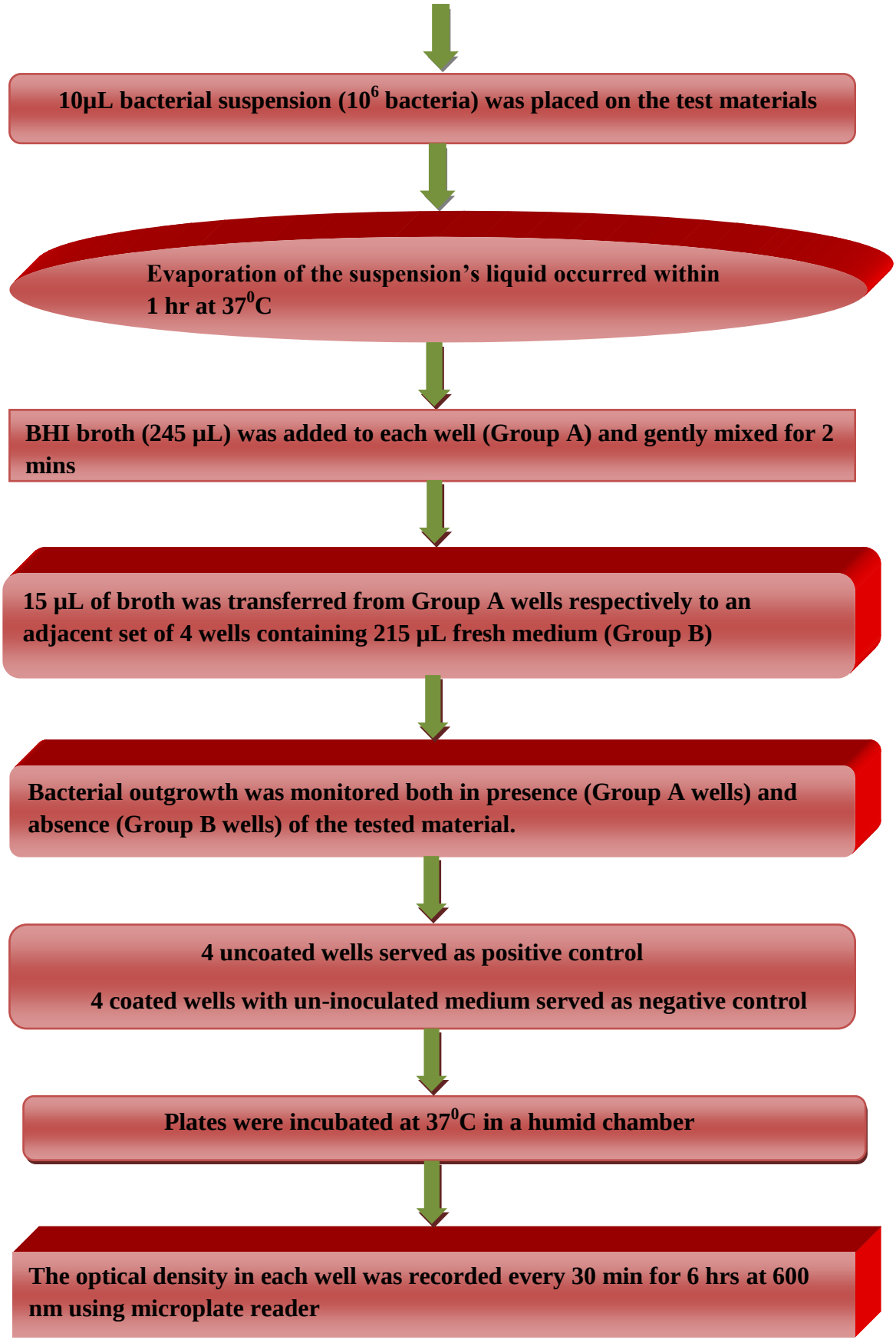
This resulted in two sets of 4 wells for each tested material containing an equal volume of liquid medium, so that bacterial outgrowth could be monitored both in the presence and in the absence of the tested material.

Two sets of 4 uncoated wells in the same microtitre plate served as positive control. In other words, identical bacterial inoculums was placed on the side wall of the uncoated wells and processed as Group A and Group B wells. The negative control consisted of a set of 4 wells coated with the tested materials as in Group A and contained an equal volume of un-inoculated fresh medium.

Plates were incubated at 37^0 C in a humid chamber. (Figure 9) Bacterial growth was followed by densitometric measurement in a microplate reader. (Figure 10) The OD in each well at 600 nm was recorded every 30 min for 6 hrs. (Figure 11) All experiments were carried out under aseptic conditions. Automixing prior to each reading ensured a homogenous bacterial cell suspension. The growth curves from Group A and B were compared with positive control outgrowth A and B respectively for all the study groups. Data were recorded; the values of the negative control wells were considered as baseline and subtracted from the respective experimental sets then plotted and statistically analyzed using ONE WAY ANOVA and TUKEY's HSD POST HOC multiple comparisons.

ANTIBACTERIAL ACTIVITY




10µL bacterial suspension (10^6 bacteria) was placed on the test materials
Evaporation of the suspension's liquid occurred within 1 hr at 37^0C
BHI broth (245 µL) was added to each well (Group A) and gently mixed for 2 mins
15 µL of broth was transferred from Group A wells respectively to an adjacent set of 4 wells containing 215 µL fresh medium (Group B)
Bacterial outgrowth was monitored both in presence (Group A wells) and absence (Group B wells) of the tested material.
4 uncoated wells served as positive control
4 coated wells with un-inoculated medium served as negative control
Plates were incubated at 37^0C in a humid chamber
The optical density in each well was recorded every 30 min for 6 hrs at 600 nm using microplate reader

ANTIBACTERIAL ACTIVITY- DIRECT CONTACT TEST

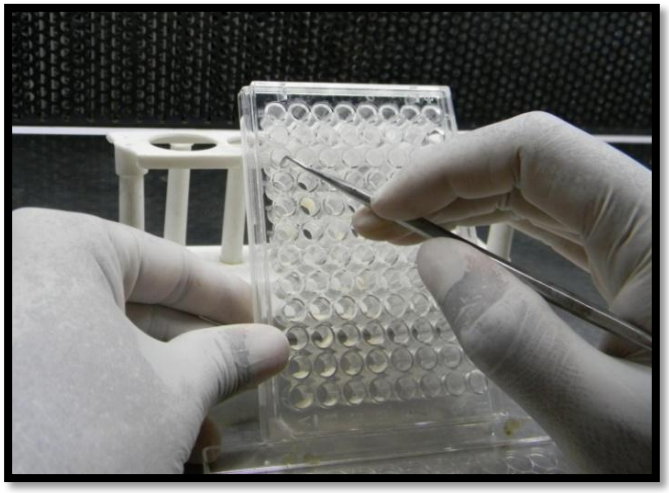


Figure 4.Coating of root canal sealers

Figure 5.Enterococcus faecalis suspension, Brain heart infusion broth

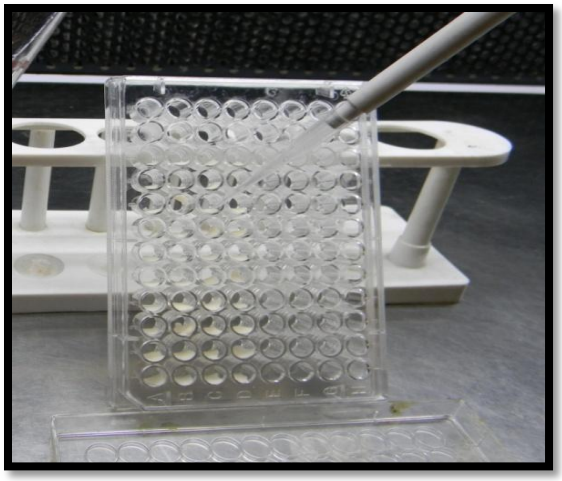


Figure 6. Placement of bacterial suspension

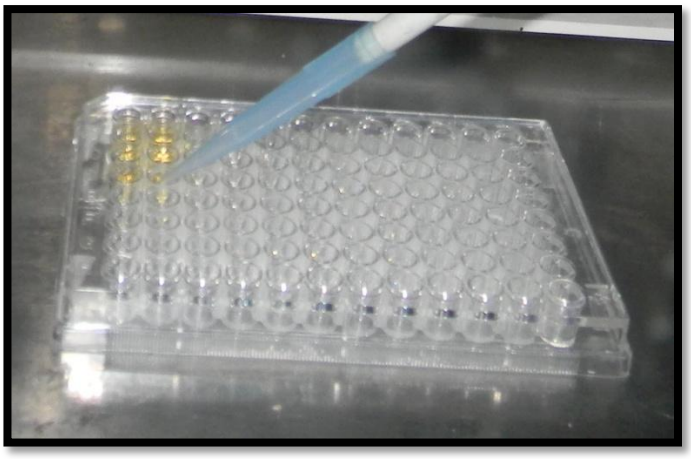


Figure 7. Adding BHI broth

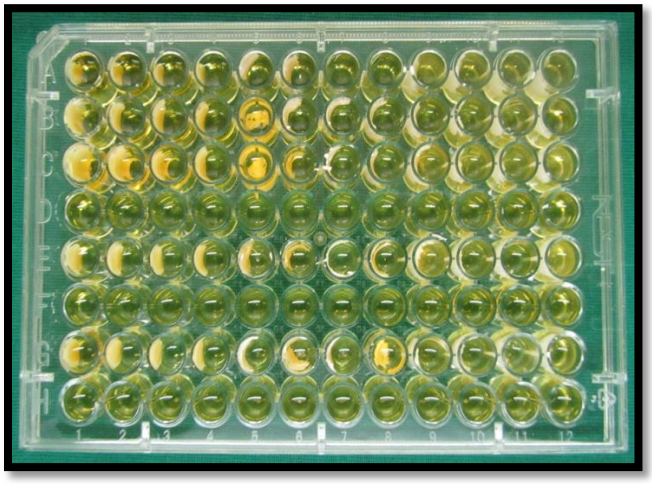


Figure 8. 96 Well Plate for Direct Contact Test

Figure 9.Incubator

Figure 10. 96 Well Microplate Reader

Figure 11. Readings Computed

FOR DENTINAL TUBULE PENETRATION DEPTH ANALYSIS BY SCANNING ELECTRON MICROSCOPE (SEM)

For decoronation of teeth

- 30 Single rooted maxillary anterior teeth
- Diamond coated disc with mandrel
- Slow speed straight handpiece (NSK)

For root canal preparation (Figure 13.)

- K –files No. 15-40 (Dentsply)
- K – files No. 45-80 (Dentsply)
- ProTaper Universal rotary system (Dentsply)
- Endogauge (Dentsply)
- Slow speed micromotor handpiece
- Anthogyr rotary handpiece
- 5% sodium hypochlorite
- RC Prep (17% EDTA)
- Distilled water
- Tweezers
- 2 ml syringe
- Burnisher
- Spirit lamp
- Light curing unit
- Paper mixing pads

- Plastic spatula
- IRM (Dentsply)
- Gutta-percha points (F_5 Dentsply)
- Paper points
- Lentulo spiral (Mani 25-40 size)

For specimen preparation

- Slow speed micromotor straight handpiece (NSK)
- Diamond coated disc
- Chisel
- Mallet

For analyzing specimens

- Scanning Electron Microscope (HITACHI S-3400N, JAPAN)
- Gold sputtering machine (E 1010 ion sputter, JAPAN)

METHODOLOGY FOR DENTINAL TUBULE PENETRATION DEPTH

30 Freshly extracted maxillary central incisiors were used for the study. (Figure 12)

INCLUSION CRITERIA:

- Single and straight canal
- Caries free teeth
- Teeth with completely formed apices

SAMPLE PROCESSING:

The teeth were rinsed under tap water in order to remove blood and tissue debris. Soft tissue tags, bone or calculus were removed and then teeth were stored in normal saline at room temperature until use.

PREPARATION OF THE SAMPLES:

30 Teeth were decoronated using diamond coated disc, under continuous water spray leaving 14 mm of root length for standardization.

Patency of the canal was established using No. 10 K file. The working length established at 1mm short of the length of the file at the point where it just exited the root.

Instrumentation was performed using K- files (Dentsply) and ProTaper Universal rotary system (S_x- F_5), RC Prep with a crown down technique (Figure 14). 5% sodium hypochlorite was used as an intermittent irrigant after each file. On completion of instrumentation, smear layer was removed by rinsing the canal with EDTA, pH: 7.3 for 1 min. Canals were ultimately rinsed with distilled water to remove all chemicals.

Master gutta-percha point (F_5) was trial fitted to achieve tug back. Each canal was then dried with paper points, randomly divided into three Groups of 10 specimens in each Group and subsequently obturated depending on the Group it belonged to.

For respective Groups sealers were mixed according to manufacturer's instructions. After drying the canal with paper points, the mixed sealers were coated into the root canals with the help of Lentulo spiral (Figure 15). The prefit master cone was then inserted into the canal to the working length.

DISTRIBUTION OF SAMPLES:

Group I (n=10): AH Plus sealer

Sealer was mixed according to manufacturer's instructions. An equal length of AH Plus paste A and paste B were dispensed on the mixing pad and mixed until uniform color was achieved.

Later, canals were obturated as described above.

Group II (n= 10): RealSeal self-etch sealer

It is a two paste system with an automix syringe, which provides a uniformly mixed sealer. Mixed sealer is coated in the root canal with the help of Lentulo spiral (size 40). After obturation, the coronal portion of the sealer was light cured for 40 sec, to stabilize the material, enabling excess gutta-percha to be removed with a hot instrument.

Group III (n= 10): iRoot SP sealer

It is a single paste system, placed in the root canal as described above and obturated. All the teeth thus obturated, coronal 2mm of the filling was removed with heated instrument for all the specimens to allow sealing the coronal end with IRM to prevent coronal leakage. All the Groups were stored in 100 % relative humidity for 7 days to ensure complete setting of the sealers.

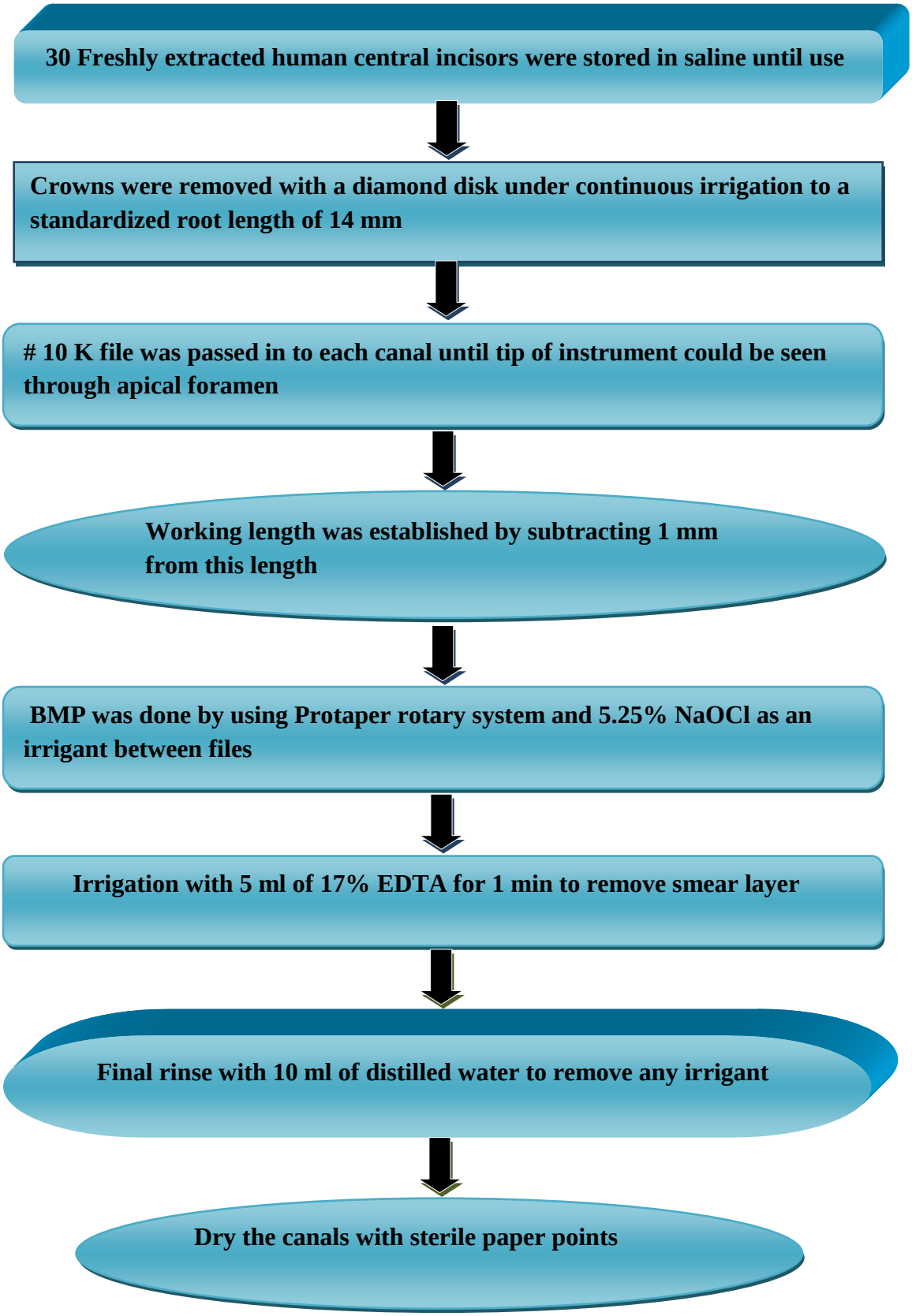
SCANNING ELECTRON MICROSCOPE EXAMINATION:

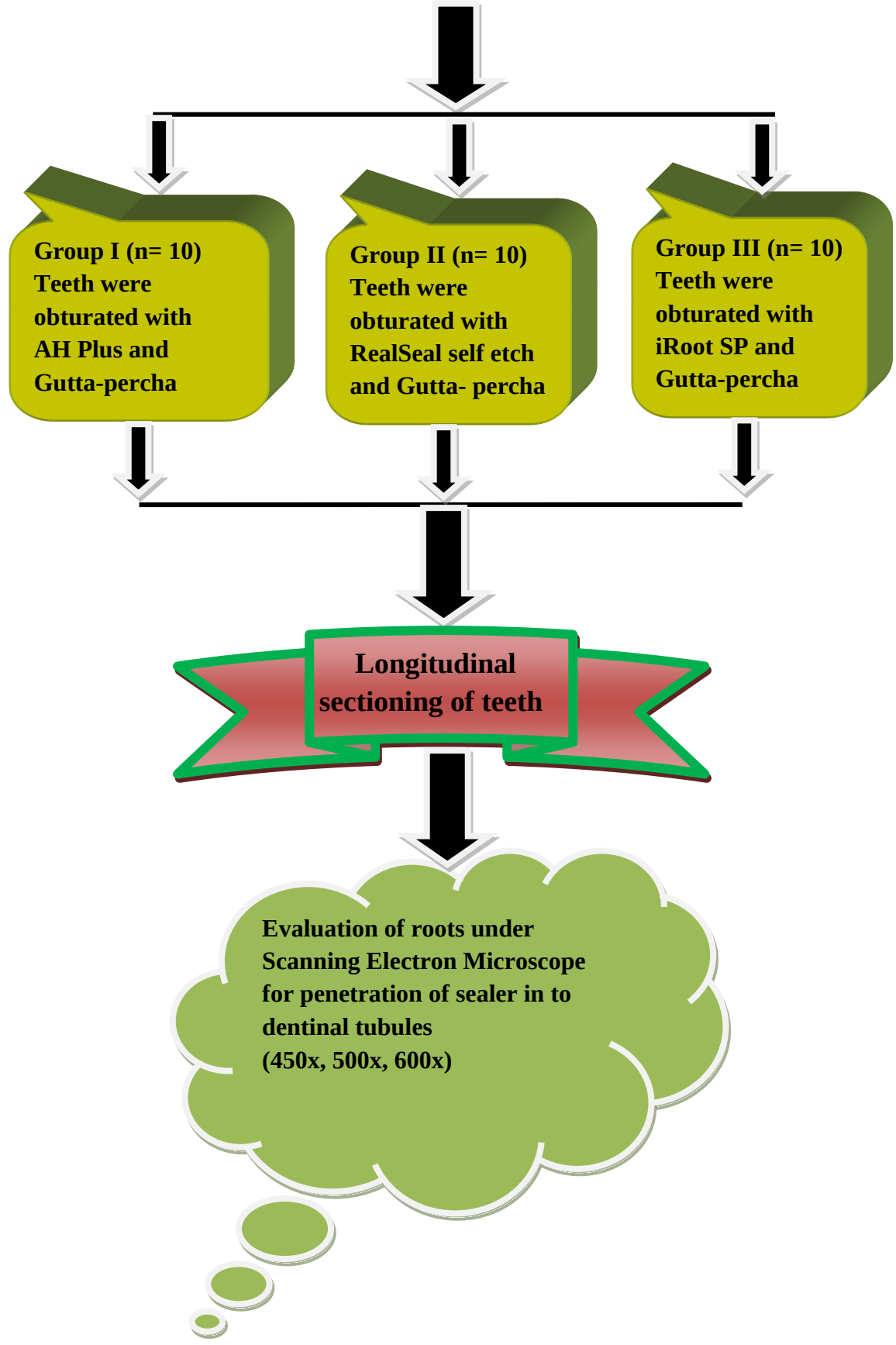
Specimens from each Group were divided into two halves by buccolingual vertical sections. Two longitudinal grooves were prepared on the buccal and lingual

surfaces of each root using a diamond disc under continuous water spray and without penetration into the canal. The roots were then split into two halves with a chisel and mallet, producing two specimens per tooth.

Three notches were made in each half using a scalpel: 4,8,12 mm apical to the most coronal level of each root. After gold sputtering the observations were made at the dentin-sealer interface under SEM (450x, 500x, 600x) at apical, middle and coronal areas of each half of root (Figure 16). Therefore, six measurements were performed in each root (Figure 17). These values were averaged to obtain a single observation for each third, for each root. The results were then tabulated and statistically analyzed using ONE WAY ANOVA and TUKEY's HSD POST HOC multiple comparisons.

PENETRATION DEPTH OF SEALERS IN DENTINAL TUBULES




Group I (n= 10) Teeth were obturated with AH Plus and Gutta-percha
Group II (n= 10) Teeth were obturated with RealSeal self etch and Gutta- percha
Group III (n= 10) Teeth were obturated with iRoot SP and Gutta-percha
Longitudinal sectioning of teeth
Evaluation of roots under Scanning Electron Microscope for penetration of sealer in to dentinal tubules (450x, 500x, 600x)

For dentinal tubule penetration depth- Scanning Electron Microscope

Figure 12. 30 Freshly extracted central incisiors

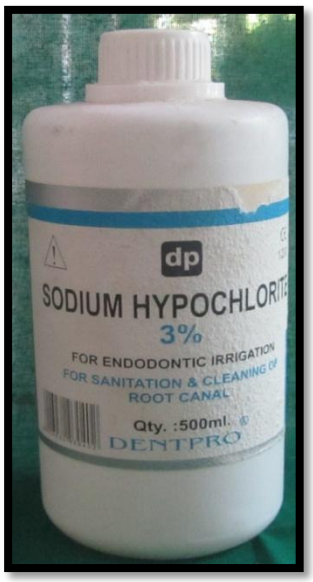


Figure 13. Armamentarium used for root canal preparation

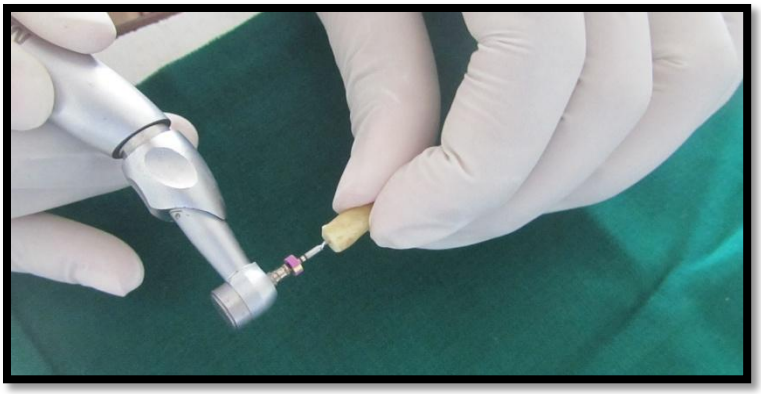


Figure 14. Root canal preparation using ProTaper Universal system

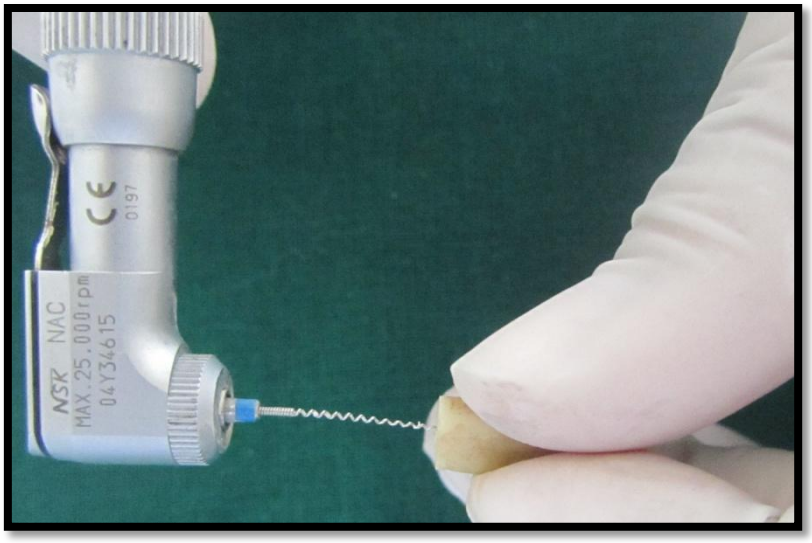


Figure 15. Application of root canal sealer with Lentulo spiral

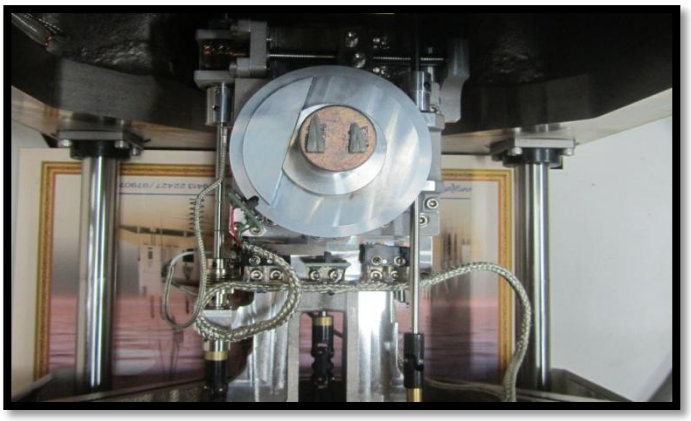


Figure 16. Loading of samples in Scanning Electron Microscope

Figure 17. Images analyzed under Scanning Electron Microscope

Results

ANTIBACTERIAL PROPERTY

The optical density (OD) readings were taken in each well for every 30 min for 6 hours. Average value was calculated from the four wells for all samples.

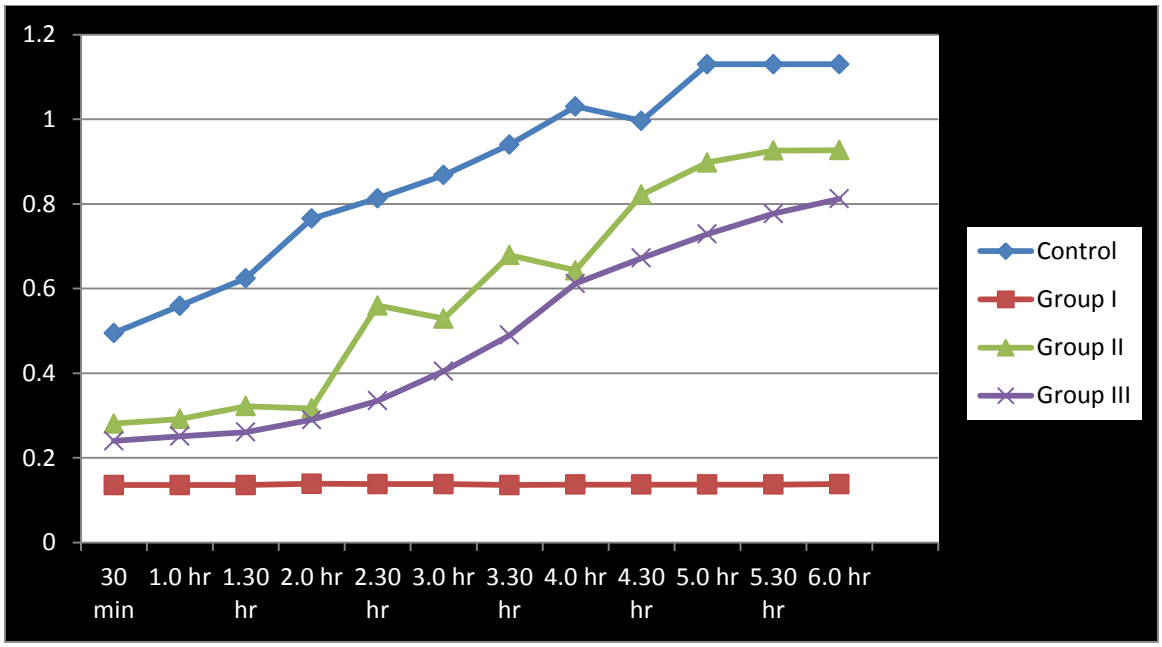
7 days sample

Table 1. Optical Density values for Group A wells of 7 days sample.

Time	Control	Group I	Group II	Group III
30 min	0.495	0.136	0.281	0.240
1.0 hr	0.559	0.136	0.292	0.251
1.30 hr	0.624	0.136	0.322	0.261
2.0 hr	0.765	0.139	0.317	0.290
2.30 hr	0.813	0.138	0.560	0.335
3.0 hr	0.868	0.138	0.529	0.405
3.30 hr	0.940	0.136	0.679	0.490
4.0 hr	1.03	0.137	0.643	0.612
4.30 hr	0.996	0.137	0.822	0.672
5.0 hr	1.13	0.137	0.898	0.729
5.30 hr	1.13	0.137	0.926	0.777
6.0 hr	1.13	0.138	0.927	0.812

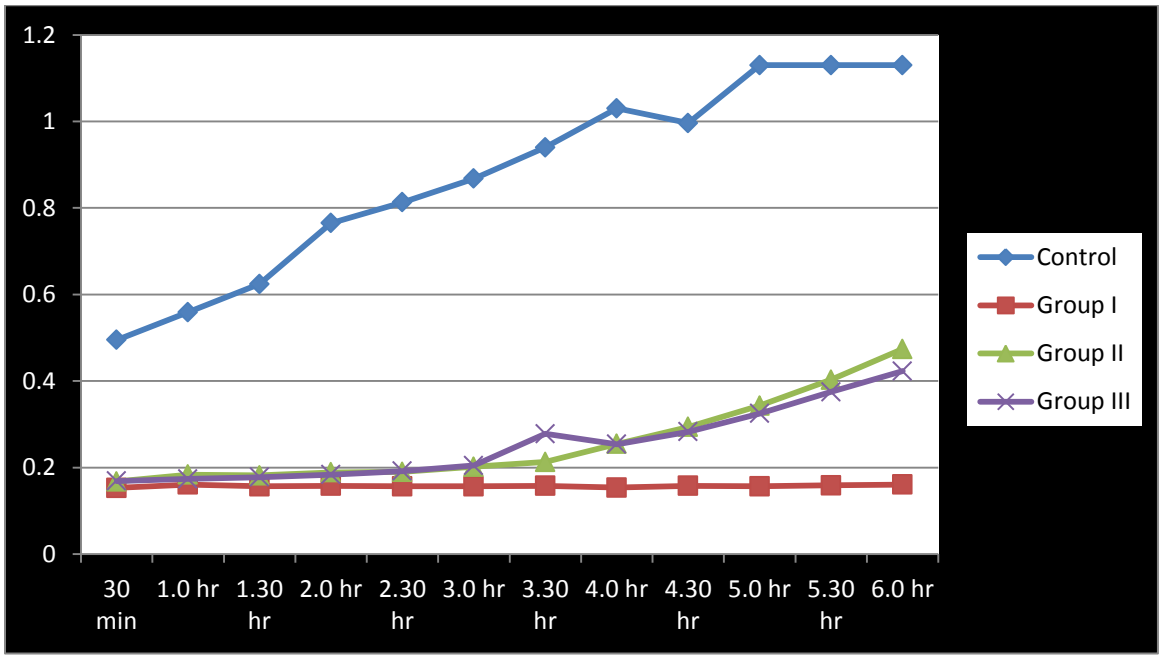
Table 2. Optical Density values for Group B wells of 7 days sample

Time	Control	Group I	Group II	Group III
30 min	0.495	0.153	0.168	0.169
1.0 hr	0.559	0.161	0.184	0.174
1.30 hr	0.624	0.157	0.182	0.178
2.0 hr	0.765	0.158	0.189	0.184
2.30 hr	0.813	0.157	0.190	0.192
3.0 hr	0.868	0.157	0.202	0.205
3.30 hr	0.940	0.158	0.213	0.278
4.0 hr	1.03	0.154	0.255	0.254
4.30 hr	0.996	0.158	0.294	0.283
5.0 hr	1.13	0.157	0.343	0.325
5.30 hr	1.13	0.159	0.403	0.375
6.0 hr	1.13	0.161	0.474	0.423



Graph 1. DCT of 7 days aged sample in the presence of material (Group A)

Each point on the growth curve is the mean of OD measurements in four wells. Each curve includes 48 measurements taken within 6 hours.



Graph 2. DCT of 7 days aged sample in the absence of material (Group B)

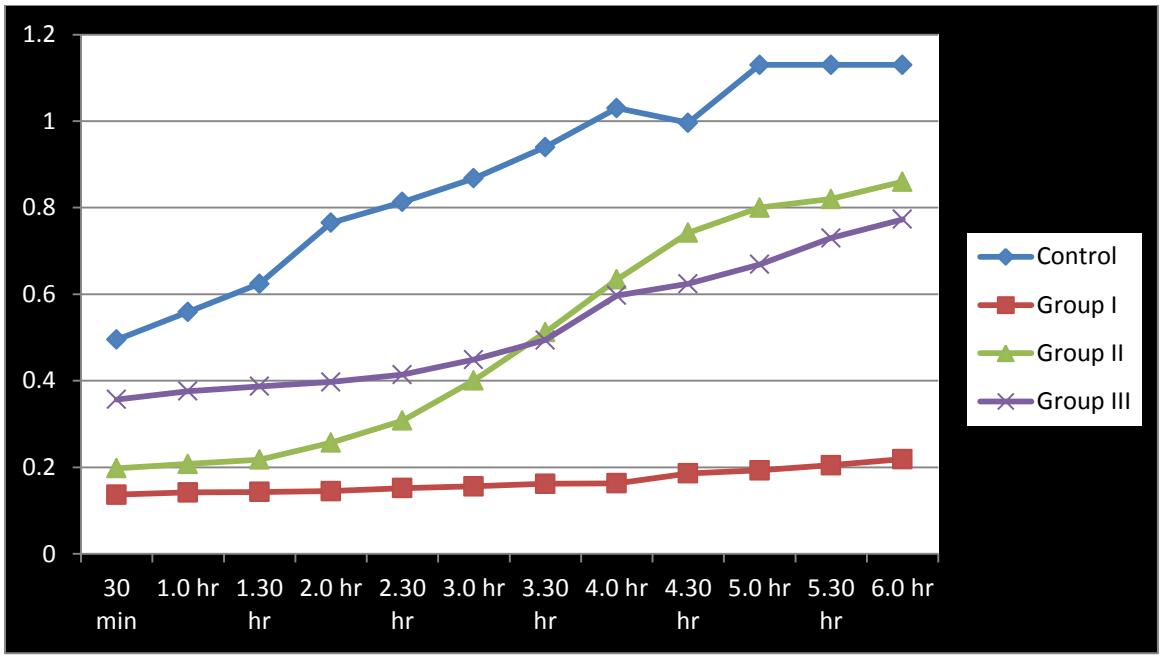
Each point on the growth curve is the mean of OD measurements in four wells. Each curve includes 48 measurements taken within 6 hours.

Table 3. Optical Density values for Group A wells of 3 days sample.

Time	Control	Group I	Group II	Group III
30 min	0.495	0.137	0.198	0.357
1.0 hr	0.559	0.142	0.208	0.376
1.30 hr	0.624	0.143	0.218	0.387
2.0 hr	0.765	0.145	0.257	0.397
2.30 hr	0.813	0.152	0.308	0.414
3.0 hr	0.868	0.156	0.401	0.449
3.30 hr	0.940	0.162	0.512	0.494
4.0 hr	1.03	0.163	0.634	0.597
4.30 hr	0.996	0.186	0.742	0.624
5.0 hr	1.13	0.193	0.800	0.669
5.30 hr	1.13	0.205	0.820	0.730
6.0 hr	1.13	0.219	0.860	0.773

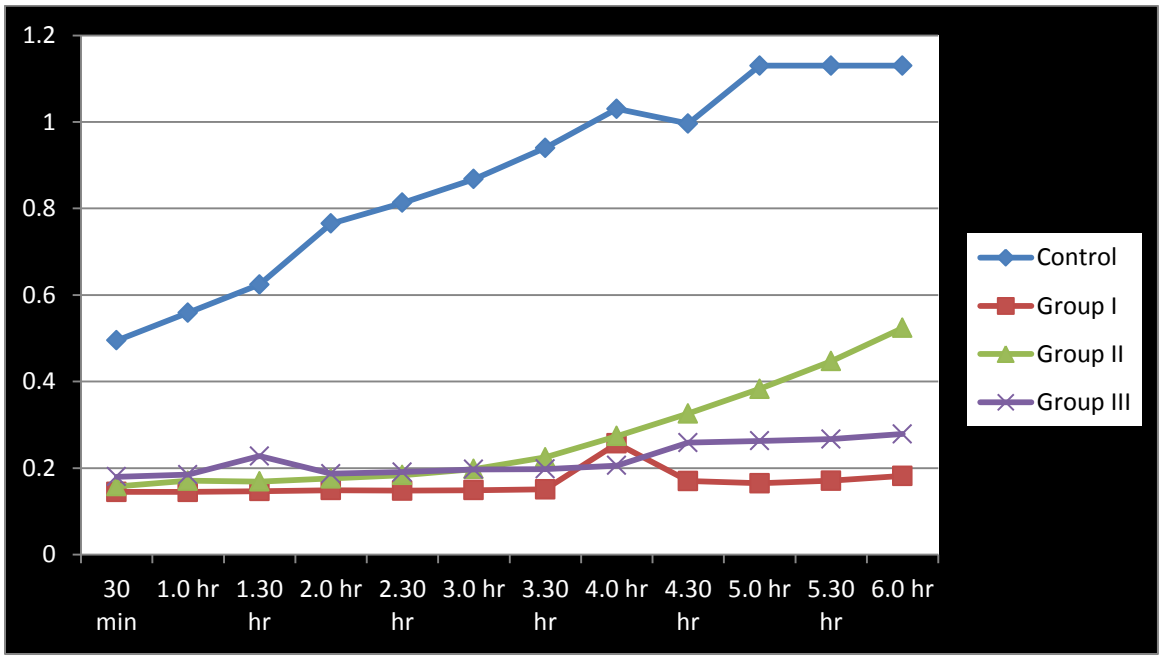
Table 4. Optical Density values for Group B wells of 3 days sample

Time	Control	Group I	Group II	Group III
30 min	0.495	0.145	0.158	0.180
1.0 hr	0.559	0.145	0.171	0.185
1.30 hr	0.624	0.147	0.169	0.228
2.0 hr	0.765	0.149	0.176	0.187
2.30 hr	0.813	0.148	0.184	0.191
3.0 hr	0.868	0.149	0.198	0.197
3.30 hr	0.940	0.151	0.225	0.198
4.0 hr	1.03	0.257	0.274	0.206
4.30 hr	0.996	0.170	0.326	0.259
5.0 hr	1.13	0.165	0.383	0.263
5.30 hr	1.13	0.171	0.447	0.267
6.0 hr	1.13	0.182	0.524	0.279



Graph 3. DCT of 3 days aged sample in the presence of material (Group A)

Each point on the growth curve is the mean of OD measurements in four wells. Each curve includes 48 measurements taken within 6 hours.



Graph 4. DCT of 3 days aged sample in the absence of material (Group B)

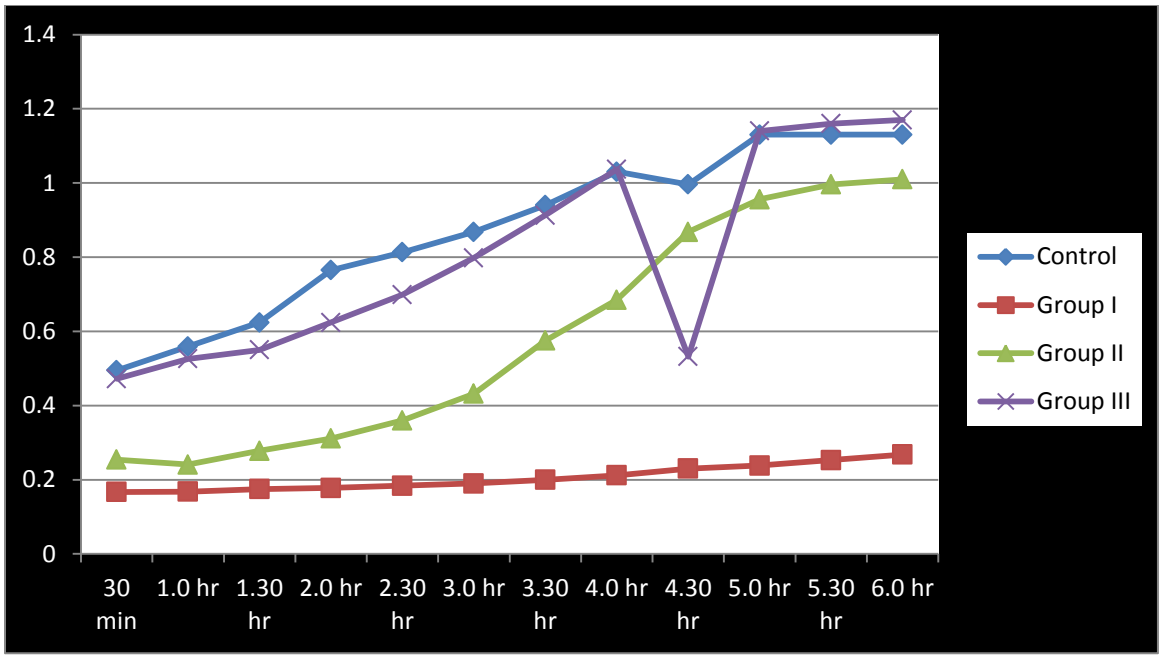
Each point on the growth curve is the mean of OD measurements in four wells. Each curve includes 48 measurements taken within 6 hours.

Table 5. Optical Density values for Group A wells of 1 day sample.

Time	**Control**	**Group I**	**Group II**	**Group III**
30 min	0.495	0.167	0.254	0.472
1.0 hr	0.559	0.168	0.241	0.526
1.30 hr	0.624	0.175	0.278	0.550
2.0 hr	0.765	0.178	0.311	0.624
2.30 hr	0.813	0.184	0.360	0.699
3.0 hr	0.868	0.190	0.432	0.798
3.30 hr	0.940	0.200	0.575	0.913
4.0 hr	1.03	0.212	0.685	1.037
4.30 hr	0.996	0.230	0.868	0.532
5.0 hr	1.13	0.238	0.956	1.14
5.30 hr	1.13	0.253	0.996	1.16
6.0 hr	1.13	0.268	1.01	1.17

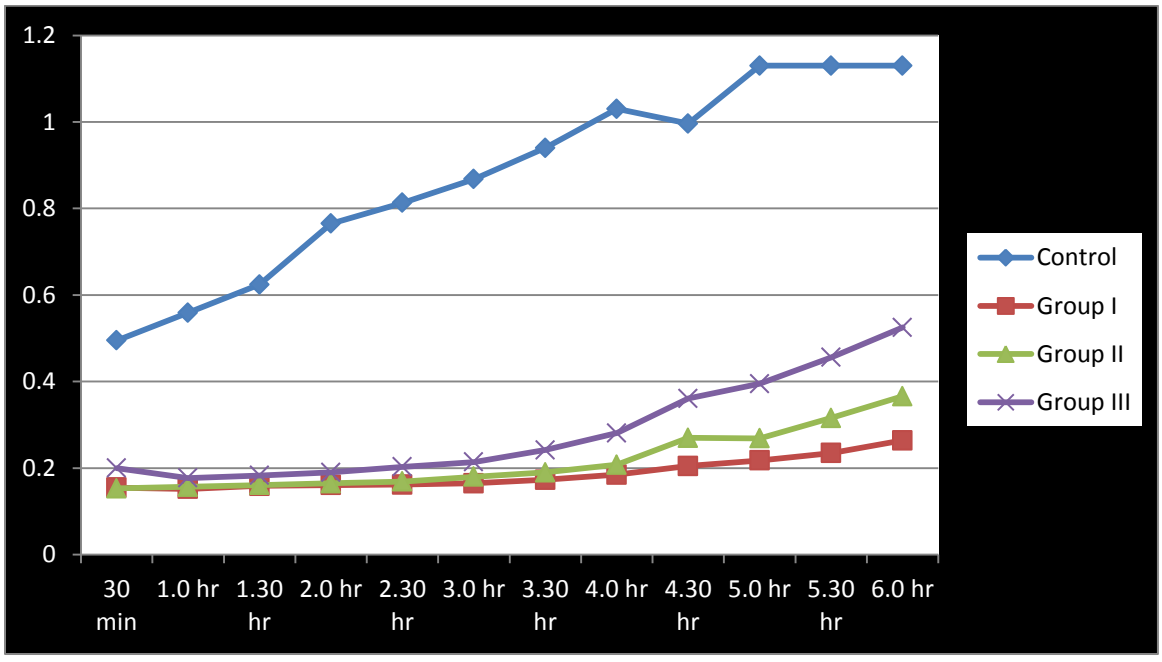
Table 6. . Optical Density values for Group B wells of 1 day sample

Time	**Control**	**Group I**	**Group II**	**Group III**
30 min	0.495	0.155	0.153	0.200
1.0 hr	0.559	0.152	0.157	0.177
1.30 hr	0.624	0.159	0.161	0.183
2.0 hr	0.765	0.161	0.165	0.190
2.30 hr	0.813	0.162	0.169	0.203
3.0 hr	0.868	0.165	0.180	0.214
3.30 hr	0.940	0.173	0.190	0.242
4.0 hr	1.03	0.185	0.208	0.281
4.30 hr	0.996	0.205	0.270	0.361
5.0 hr	1.13	0.218	0.269	0.395
5.30 hr	1.13	0.235	0.316	0.456
6.0 hr	1.13	0.264	0.366	0.525



Graph 5. DCT of 1 day aged sample in the presence of material (Group A)

Each point on the growth curve is the mean of OD measurements in four wells. Each curve includes 48 measurements taken within 6 hours.



Graph 6. DCT of 1 day aged sample in the absence of material (Group B)

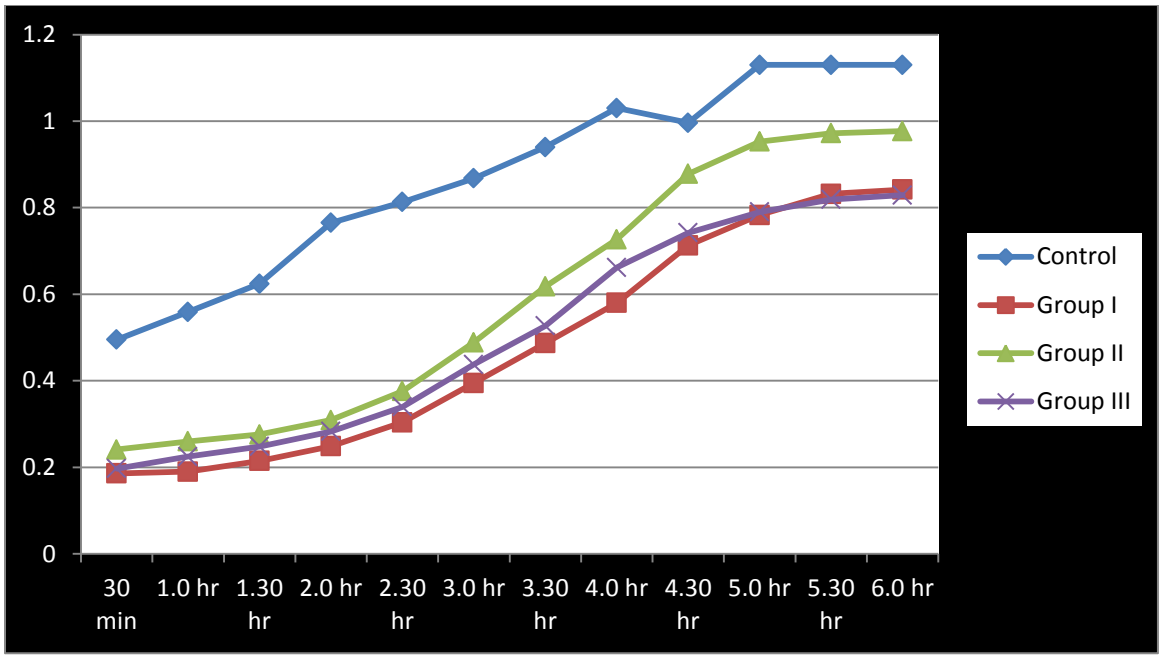
Each point on the growth curve is the mean of OD measurements in four wells. Each curve includes 48 measurements taken within 6 hours.

Table 7. Optical Density values for Group A wells of fresh sample

Time	**Control**	**Group I**	**Group II**	**Group III**
30 min	0.495	0.186	0.241	0.197
1.0 hr	0.559	0.190	0.260	0.225
1.30 hr	0.624	0.215	0.276	0.248
2.0 hr	0.765	0.249	0.309	0.283
2.30 hr	0.813	0.304	0.376	0.340
3.0 hr	0.868	0.395	0.489	0.438
3.30 hr	0.940	0.487	0.618	0.527
4.0 hr	1.03	0.580	0.727	0.662
4.30 hr	0.996	0.713	0.878	0.742
5.0 hr	1.13	0.783	0.953	0.790
5.30 hr	1.13	0.832	0.972	0.819
6.0 hr	1.13	0.842	0.977	0.829

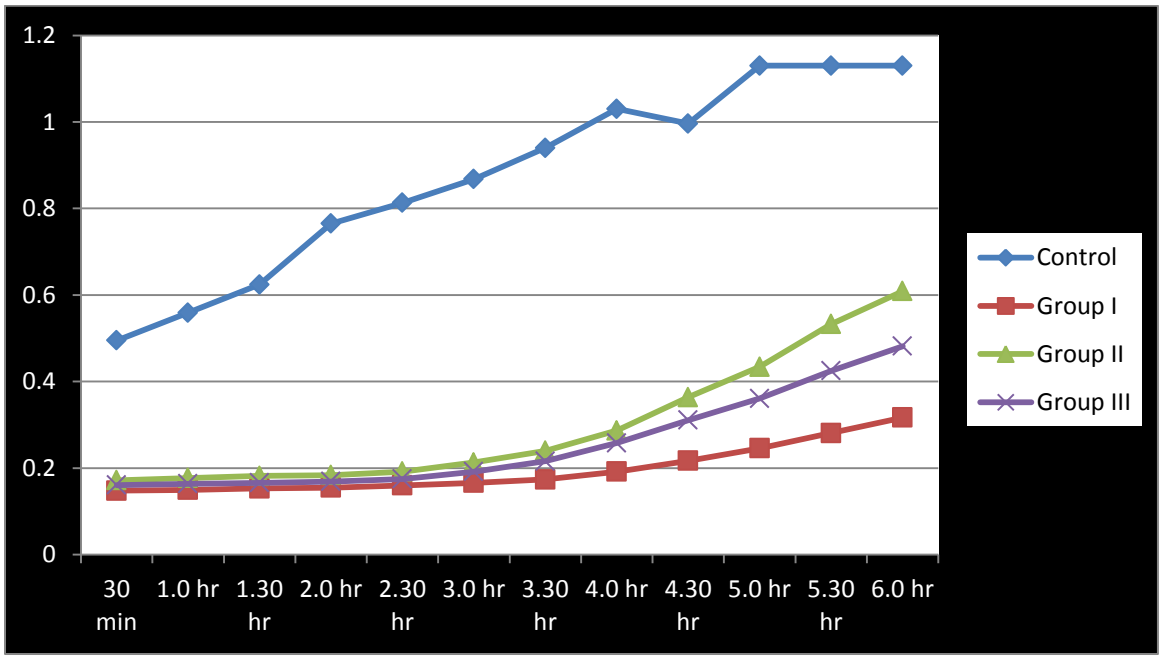
Table 8. Optical Density values for Group B wells of fresh sample

Time	**Control**	**Group I**	**Group II**	**Group III**
30 min	0.495	0.148	0.172	0.161
1.0 hr	0.559	0.150	0.177	0.164
1.30 hr	0.624	0.153	0.182	0.166
2.0 hr	0.765	0.155	0.184	0.169
2.30 hr	0.813	0.160	0.192	0.175
3.0 hr	0.868	0.166	0.213	0.192
3.30 hr	0.940	0.174	0.240	0.216
4.0 hr	1.03	0.192	0.287	0.258
4.30 hr	0.996	0.217	0.364	0.311
5.0 hr	1.13	0.246	0.434	0.361
5.30 hr	1.13	0.281	0.533	0.425
6.0 hr	1.13	0.317	0.609	0.482



Graph 7. DCT of freshly mixed sample in the presence of material (Group A)

Each point on the growth curve is the mean of OD measurements in four wells. Each curve includes 48 measurements taken within 6 hours.



Graph 8. DCT of freshly mixed sample in the absence of material (Group B)

Each point on the growth curve is the mean of OD measurements in four wells. Each curve includes 48 measurements taken within 6 hours.

STATISTICAL ANALYSIS

Data were statistically analyzed using **ONE WAY ANOVA** and **TUKEY's HSD POST HOC** multiple comparisons at 0 .05 level significance.

Table 9. **ONE WAY ANOVA** for Group A values of DCT

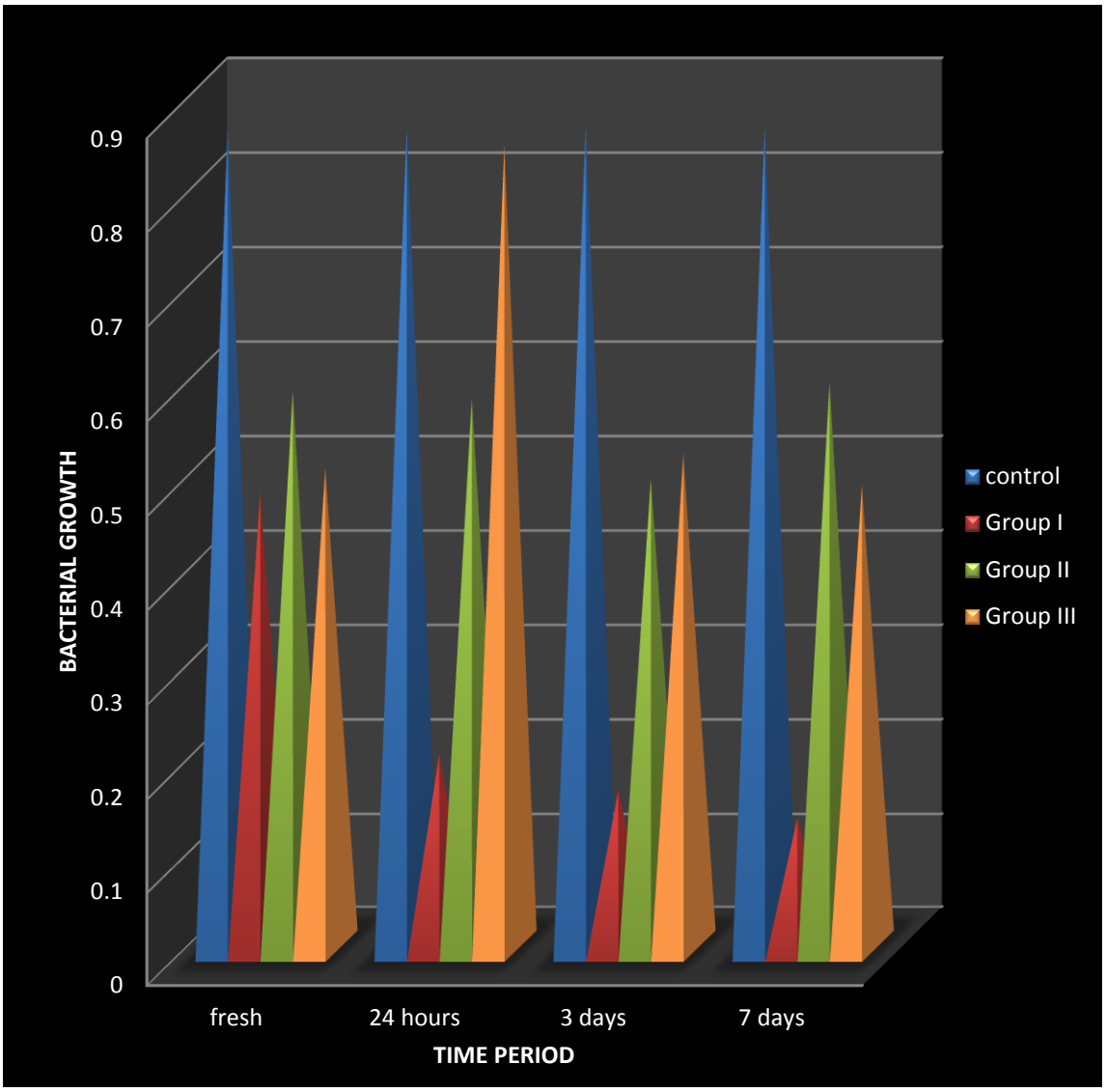
	group	mean	Standard deviation	significance
7 DAYS	control	0.869	.288	.000
	Group I	0.138	.024	.000
	Group II	0.600	.282	.000
	Group III	0.490	.233	.000
3 DAYS	control	0.869	.288	.000
	Group I	0.167	.039	.000
	Group II	0.497	.272	.000
	Group III	0.523	.295	.000
24 HOURS	control	0.869	.288	.000
	Group I	0.206	.073	.000
	Group II	0.582	.306	.000
	Group III	0.851	.312	.000
FRESH SAMPLE	control	0.869	.288	.000
	Group I	0.482	.311	.000
	Group II	0.590	.294	.000
	Group III	0.509	.285	.000

Table 10. **TUKEY's HSD POST HOC** for Group A values of DCT

Dependant variable	(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
7 days	Control	Group I	.73223(*)	.047682	.000	.60863	.85583
		Group II	.26958(*)	.047682	.000	.14598	.39318
		Group III	.37981(*)	.047682	.000	.25621	.50341
	Group I	Control	-.73223(*)	.047682	.000	-.85583	-.60863
		Group II	-.46265(*)	.047682	.000	-.58625	-.33905
		Group III	-.35242(*)	.047682	.000	-.47602	-.22882
	Group II	Control	-.26958(*)	.047682	.000	-.39318	-.14598
		Group I	.46265(*)	.047682	.000	.33905	.58625
		Group III	.11023	.047682	.099	-.01337	.23383
	Group III	Control	-.37981(*)	.047682	.000	-.50341	-.25621
		Group I	.35242(*)	.047682	.000	.22882	.47602
		Group II	-.11023	.047682	.099	-.23383	.01337

	(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
3 days	Control	Group I	.70256(*)	.050672	.000	.57121	.83391
		Group II	.37292(*)	.050672	.000	.24157	.50427
		Group III	.34725(*)	.050672	.000	.21590	.47860
	Group I	Control	-.70256(*)	.050672	.000	-.83391	-.57121
		Group II	-.32965(*)	.050672	.000	-.46099	-.19830
		Group III	-.35531(*)	.050672	.000	-.48666	-.22396
	Group II	Control	-.37292(*)	.050672	.000	-.50427	-.24157
		Group I	.32965(*)	.050672	.000	.19830	.46099
		Group III	-.02567	.050672	.957	-.15702	.10568
	Group III	Control	-.34725(*)	.050672	.000	-.47860	-.21590
		Group I	.35531(*)	.050672	.000	.22396	.48666
		Group II	.02567	.050672	.957	-.10568	.15702
1 day	Control	Group I	.66408(*)	.054017	.000	.52406	.80410
		Group II	.28829(*)	.054017	.000	.14827	.42831
		Group III	.01858	.054017	.986	-.12144	.15860
	Group I	Control	-.66408(*)	.054017	.000	-.80410	-.52406
		Group II	-.37579(*)	.054017	.000	-.51581	-.23577
		Group III	-.64550(*)	.054017	.000	-.78552	-.50548
	Group II	Control	-.28829(*)	.054017	.000	-.42831	-.14827
		Group I	.37579(*)	.054017	.000	.23577	.51581
		Group III	-.26971(*)	.054017	.000	-.40973	-.12969
	Group III	Control	-.01858	.054017	.986	-.15860	.12144
		Group I	.64550(*)	.054017	.000	.50548	.78552
		Group II	.26971(*)	.054017	.000	.12969	.40973
Fresh sample	Control	Group I	.38815(*)	.060204	.000	.23209	.54421
		Group II	.27969(*)	.060204	.000	.12363	.43575
		Group III	.36117(*)	.060204	.000	.20511	.51723
	Group I	Control	-.38815(*)	.060204	.000	-.54421	-.23209
		Group II	-.10846	.060204	.276	-.26452	.04760
		Group III	-.02698	.060204	.970	-.18304	.12908
	Group II	Control	-.27969(*)	.060204	.000	-.43575	-.12363
		Group I	.10846	.060204	.276	-.04760	.26452
		Group III	.08148	.060204	.530	-.07458	.23754
	Group III	Control	-.36117(*)	.060204	.000	-.51723	-.20511
		Group I	.02698	.060204	.970	-.12908	.18304
		Group II	-.08148	.060204	.530	-.23754	.07458

* The mean difference is significant at the 0.05 level.



Graph 9. Histogram representation of survival of E faecalis after Direct Contact with 7 days, 3 days, 24 hrs. and freshly set test materials.

Interpretation of results of Direct Contact Test

Analysis of the results of DCT at 0.05 level significance reveals that

Bacterial growth was reduced significantly when compared to control in **Group I (AH Plus), Group II (RealSeal self-etch) and Group III (iRoot SP)** in both Group A and Group B wells i.e. in the presence and absence of the test material for 7 days, 3 days, 24 hrs and fresh samples.

For **fresh samples** there is no significant difference in antibacterial property between Group I (AH Plus), Group II (RealSeal self-etch) and Group III (iRoot SP).

For **24 hrs** set samples antibacterial property:

Group I (AH Plus) > Group II (RealSeal self-etch) > Group III (iRoot SP)

For **3 day** set samples antibacterial property:

Group I (AH Plus) > Group II (RealSeal self-etch) ≥ Group III (iRoot SP)

For **7 day** set samples antibacterial property:

Group I (AH Plus) > Group III (iRoot SP) ≥ Group II (RealSeal self-etch)

DENTINAL TUBULE PENETRATION DEPTH

The penetration depth of root canal sealers measured in coronal third, middle third and apical third of root canal at various magnifications (450x, 500x, 600x).

Table11. Penetration depth of root canal sealers in dentinal tubules at coronal third of root canal in **µm**.

GROUP I	GROUP II	GROUP III
40.2	48.4	108
42.8	87.9	94.6
75.8	83.1	101
75.7	81.4	152
67.5	68	152
69.4	67.6	99.25
65.88	50.4	84.12
56.44	49.8	97.25
75.44	49.6	102.75
79.77	49.1	94.75

Table12. Penetration depth of root canal sealers in dentinal tubules at middle third of root canal in **µm**.

GROUP I	GROUP II	GROUP III
38.2	57.7	99.9
57.6	71.5	91.8
39.9	40.6	95.6
36.5	36.8	94.8
32.5	36	83.9
64.14	54.7	46.7
43.73	58.2	96.5
31.17	58.1	85.87
66.25	54.1	78.37
54.37	60.8	88.9

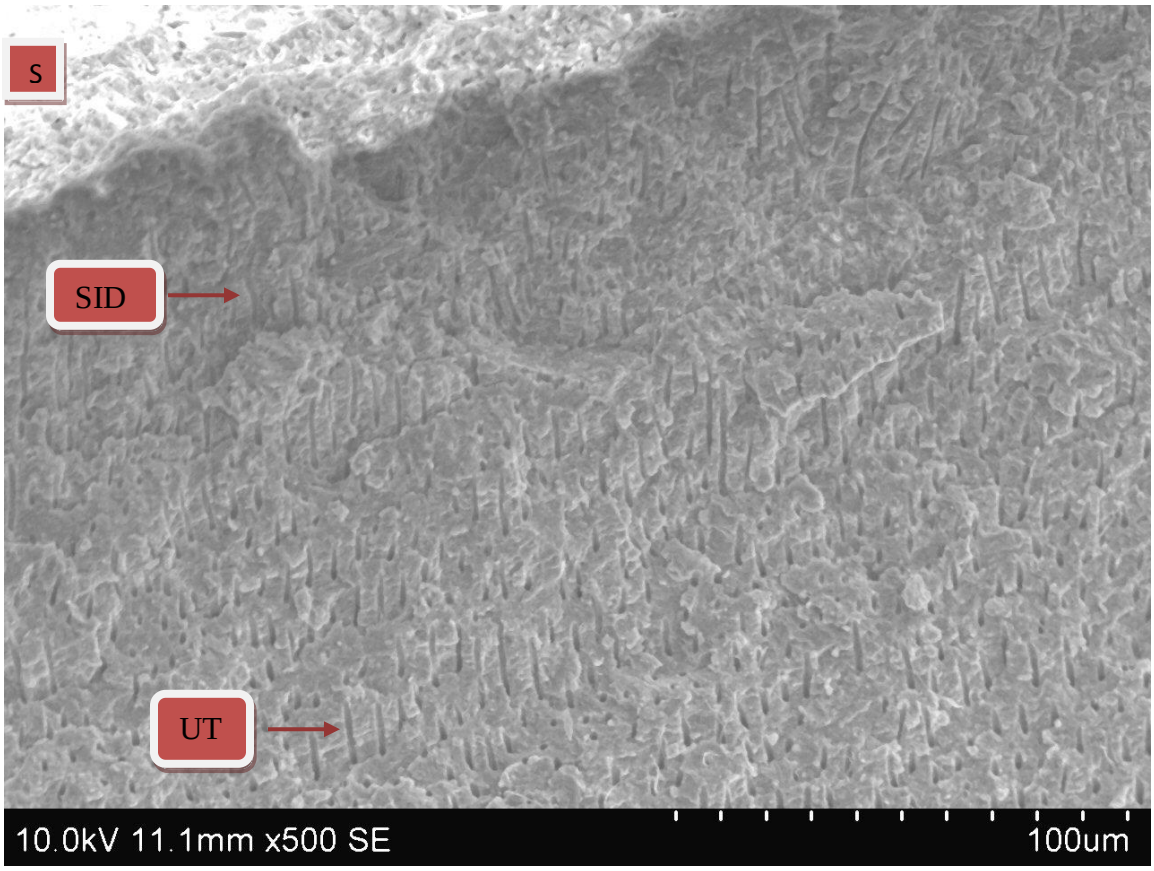
Table13. Penetration depth of root canal sealers in dentinal tubules at apical third of root canal in **µm**.

GROUP I	GROUP II	GROUP III
46.2	30	65.6
31	30.6	64.9
45.8	54.4	62.9
36.3	45.6	57.4
37.2	43.4	57.7
52.3	46.6	47.0
55	47.6	52.9
29.4	38.4	62.3
29.1	52.2	71.2
31.6	49.0	45.5

SCANNING ELECTRON MICROSCOPE (SEM) IMAGES

Group I **(AH Plus)** root canal sealer

Maximum mean penetration depth in dentinal tubules= **64.89µm**



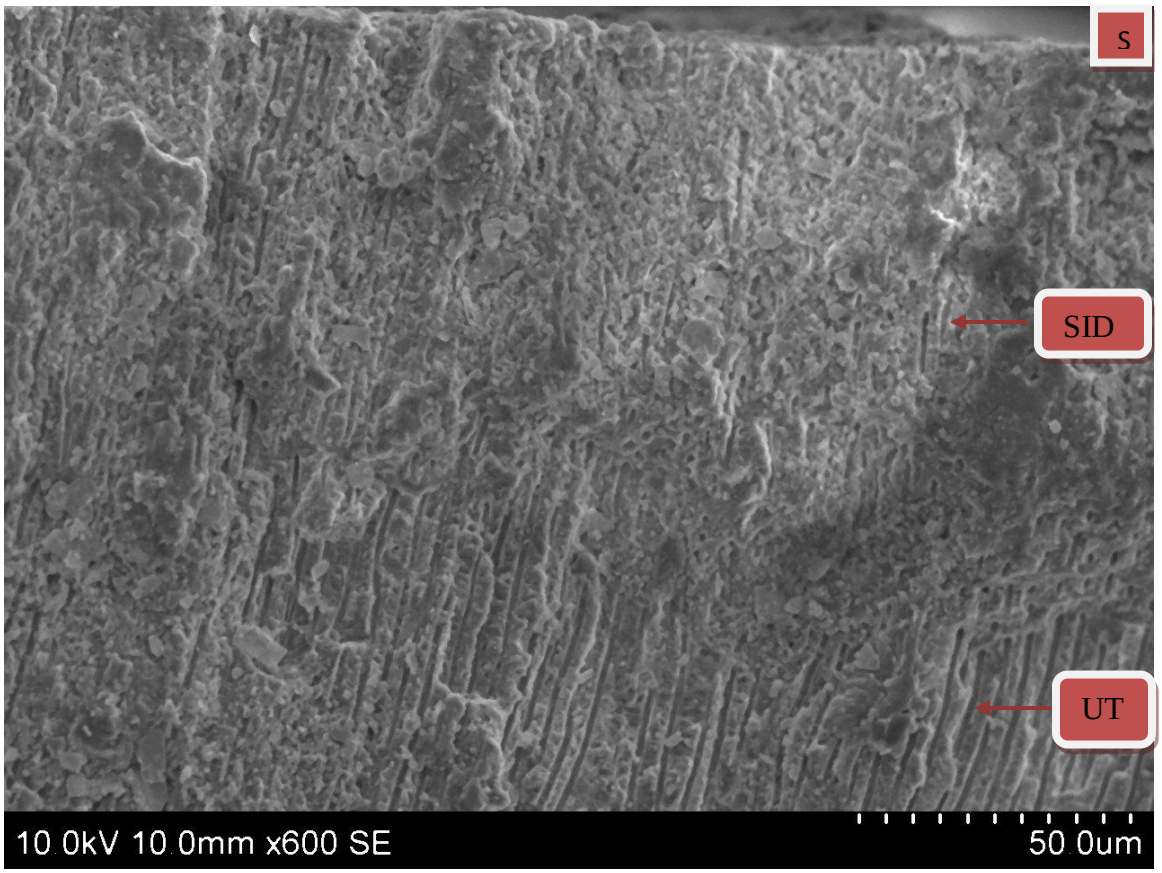
S → **ROOT CANAL SEALER**

SID → **ROOT CANAL SEALER IN DENTINAL TUBULES**

UT → **UNFILLED DENTINAL TUBULES**

Group II **(RealSeal self-etch)** root canal sealer

Maximum mean penetration depth in dentinal tubules = **63.53µm**



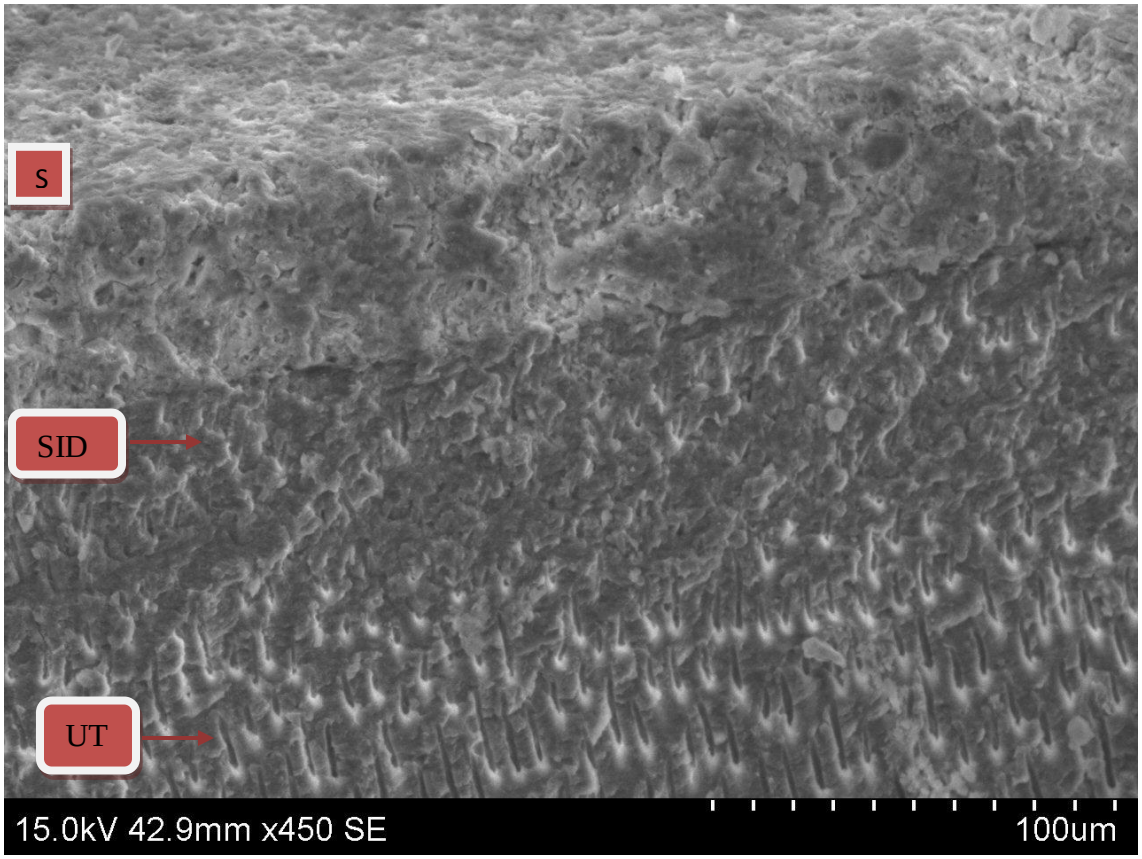
S → **ROOT CANAL SEALER**

SID → **ROOT CANAL SEALER IN DENTINAL TUBULES**

UT → **UNFILLED DENTINAL TUBULES**

Group III **(iRoot SP)** root canal sealer

Maximum mean penetration depth in dentinal tubules=**108.57µm**



S → **ROOT CANAL SEALER**

SID → **ROOT CANAL SEALER IN DENTINAL TUBULES**

UT → **UNFILLED DENTINAL TUBULES**

STATISTICAL ANALYSIS

The penetration depth of root canal sealers measured in coronal third, middle third and apical third of root canal at various magnifications (450x, 500x, 600x) and analyzed using ONE WAY ANOVA and TUKEY's HSD POST HOC multiple comparisons at 0.05 level significance.

Table 14. **ONE WAY ANOVA**

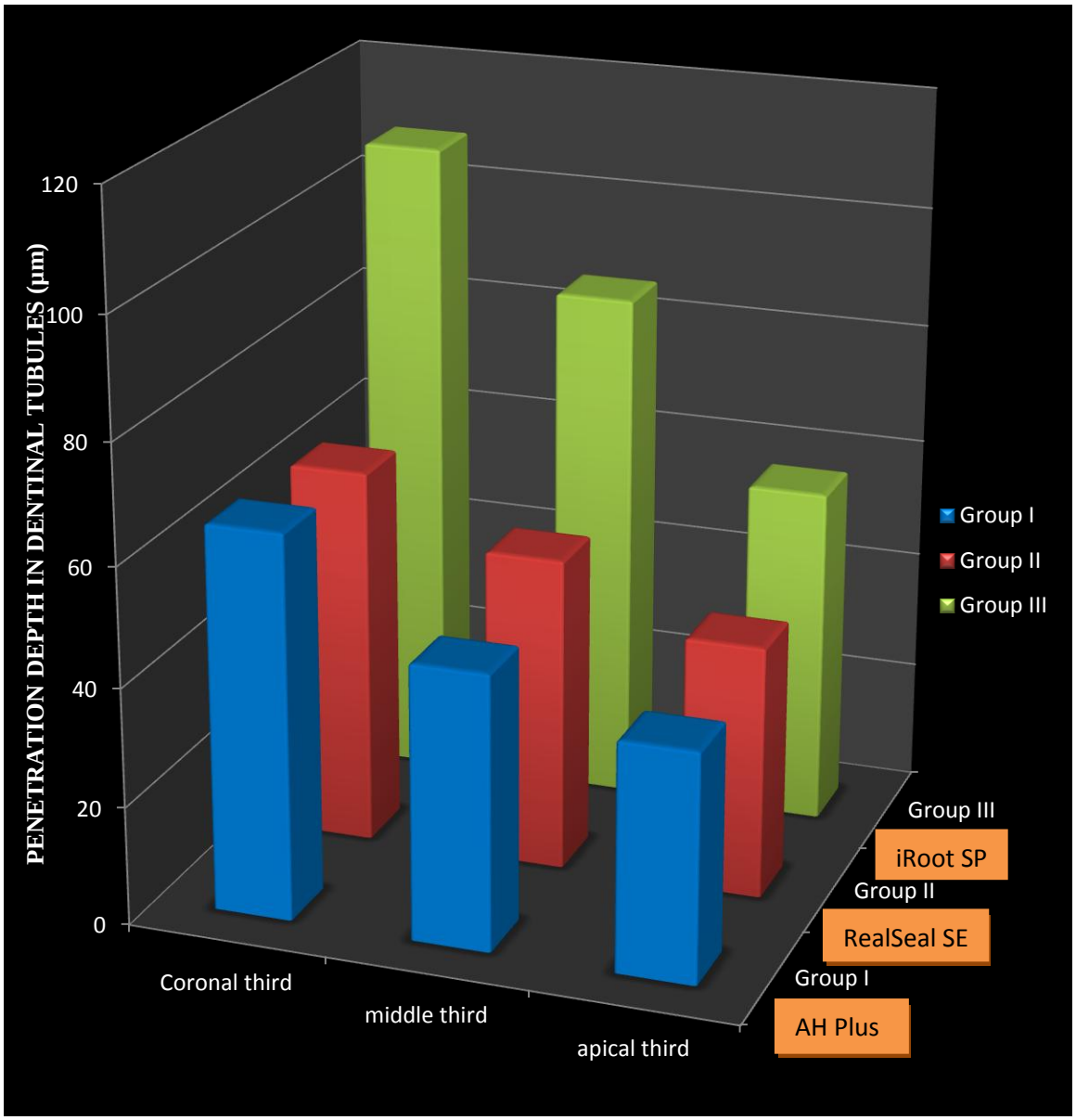
		Sum of Squares	df	Mean Square	F	Sig.
CORONAL THIRD	Between Groups	13128.315	2	6564.158	19.363	.000
	Within Groups	9153.354	27	339.013		
	Total	22281.669	29			
MIDDLE THIRD	Between Groups	9131.706	2	4565.853	25.437	.000
	Within Groups	4846.471	27	179.499		
	Total	13978.177	29			
APICAL THIRD	Between Groups	1836.254	2	918.127	10.104	.001
	Within Groups	2453.529	27	90.871		
	Total	4289.783	29			

Table 15**.TUKEY's HSD POST HOC TEST**

Dependent Variable	(I) GROUPS	(J) GROUPS	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
CORONAL THIRD	Group I	Group II	1.3630	8.23423	.985	-19.0531	21.7791
		Group III	43.6790(*)	8.23423	.000	-	-

						64.0951	23.2629
	Group II	Group I	-1.3630	8.23423	.985	-21.7791	19.0531
		Group III	45.0420(*)	8.23423	.000	-65.4581	-24.6259
	Group III	Group I	43.6790(*)	8.23423	.000	23.2629	64.0951
		Group II	45.0420(*)	8.23423	.000	24.6259	65.4581
MIDDLE THIRD	Group I	Group II	-6.4140	5.99164	.540	-21.2698	8.4418
		Group III	-39.7980(*)	5.99164	.000	-54.6538	-24.9422
	Group II	Group I	6.4140	5.99164	.540	-8.4418	21.2698
		Group III	-33.3840(*)	5.99164	.000	-48.2398	-18.5282
	Group III	Group I	39.7980(*)	5.99164	.000	24.9422	54.6538
		Group II	33.3840(*)	5.99164	.000	18.5282	48.2398
APICAL THIRD	Group I	Group II	-4.3900	4.26313	.565	-14.9601	6.1801
		Group III	-18.3500(*)	4.26313	.001	-28.9201	-7.7799
	Group II	Group I	4.3900	4.26313	.565	-6.1801	14.9601
		Group III	-13.9600(*)	4.26313	.008	-24.5301	-3.3899
	Group III	Group I	18.3500(*)	4.26313	.001	7.7799	28.9201
		Group II	13.9600(*)	4.26313	.008	3.3899	24.5301

* The mean difference is significant at the .05 level.



Graph 10. Histogram showing comparison of dentinal tubule penetration depth between various groups at Coronal, Middle and Apical third.

INTERPRETATION OF RESULTS:

Penetration depth into dentinal tubules is significantly greater in **Group III (iRoot SP) (**108.57µm) as compared to **Group I (AH Plus) (**64.89µm) and **Group II (RealSeal self-etch) (**63.53µm). There is no statistically significant difference in penetration depth between **Group I (AH Plus)** and **Group II (RealSeal self-etch).**

Discussion

Three dimensional fluid tight seal of the root canal system is the main aim of endodontic treatment and is essential for prevention of canal re-infection and maintenance of healthy periapical tissues.

Microbes and microbial products are the primary etiological agents responsible for causing periapical disease. Antibacterial activity of sealer might help to eliminate residual microorganisms that have survived the chemomechanical preparation and thereby improve the success rate of endodontic treatment[28].

AH Plus is an epoxy resin-based root canal sealer, having very good physicochemical properties. Various studies has been done on AH Plus regarding its antimicrobial property and dentinal tubule penetration depth, and it provides a satisfactory results in clinical situations. Therefore, this root canal sealer has been used as a standard to compare with another two materials in the present study.

To prevent the microleakage and re-infection in the root canal, monoblock concept using resin-based root canal sealers has been introduced. Another advantage of monoblock concept is to strengthen the canal. In three generations of resin-based root canal sealers a separate etching agent and primer was required to achieve the monoblock. This makes the procedure technique sensitive and time consuming. To overcome all these limitations recently, a fourth generation resin based sealers has been introduced which includes Epiphany self-etch, RealSeal self-etch and Metaseal. There are no studies in literature related to antibacterial activity and dentinal tubule penetration depth of RealSeal self-etch sealer. Hence, this root canal sealer has been chosen for the present study.

Bioceramics have been widely used in medicine and dentistry because of its non-toxic nature and biocompatibility. Other advantages of bioceramics are that they do not shrink, chemically stable within biological environment and will not provoke any inflammatory reaction after coming in direct contact with living tissues.

Bioceramics such as calcium-phosphate based materials have already been used in endodontics for repair and apical retrofills e.g. MTA (Tulsa Dentsply) and Bioaggregate (DiaDent).

Recently, a new bioceramic based premixed root canal sealer EndoSequence BC and iRoot SP, which hardens only when exposed to moisture has been introduced. Dentin contains water of approximately 20 percent by volume. According to the manufacturer this new bioceramic root canal sealer utilizes this water to set and ultimately forms calcium silicate hydrate gel and hyroxyapatite. All these features can provide a promising effect on the overall success of root canal treatment. There is very little information available regarding physiochemical properties of iRoot SP root canal sealer. Hence, this new bioceramic based root canal sealer iRoot SP has been evaluated and compared to standardize root canal sealer AH Plus.

ANTIBACTERIAL ACTIVITY

The **Direct Contact Test** (DCT) described by **Wiess et al (1996)**[12], was performed in this study is to date considered a valuable in vitro assay to study the antimicrobial properties of solid dental materials. The DCT is based on measuring the effect of close and direct contact between the test bacteria and tested material on kinetics

of bacterial outgrowth using a microplate spectrophotometer regardless of the solubility and diffusibility of antimicrobial components of the tested material.

The experimental setup of DCT attempted to overcome some of the limitations of Agar Diffusion Test. In addition to its reproducible and quantitative nature, the results of DCT, unlike those of Agar Diffusion Test, were not sensitive to the size of inoculums. Another aspect of the setup of the test included the ability to follow bacterial growth, both in the presence (Group A) and absence of the tested materials (Group B) [12].

Following the outgrowth of test microorganism in the presence of tested material (Group A wells) is equivalent to measuring both the direct contact effect and effect of those components which are capable of diffusing into liquid medium. On the other hand, following bacterial growth in the absence of tested materials (Group B wells) measures the effect of direct contact incubation period only.

In this study bacterial growth was monitored for 6 hours, since after inoculation the exponential growth phase of the bacteria is achieved during 6 hours of incubation and thereafter the growth becomes static.

A drawback of DCT was the exposure of microtiter plate during the experimental setup to different environments, thus increasing the chance for contamination. To prevent contamination all experiments were carried out under strict aseptic conditions and negative control was maintained in each plate which contained an equal volume of un-inoculated fresh medium and monitored.

E. faecalis was chosen for the study because of its presence in association with persistent apical periodontitis[25], difficult elimination from the root canal with use of chemomechanical procedures[14], high resistance to a wide range of microbial agents[27], and

for ease in culturing and manipulation[40]. In fact, failed root canal treatment cases are nine times more likely to contain E. faecalis than primary infections. It can survive with only scant amounts of substrate and without the support of other microorganisms, and then grow to establish mono-infections which are difficult to eradicate using conventional root canal procedures[61]. E.faecalis resists destruction by forming a biofilm which is 1000 times more resistant to phagocytosis, antibodies and antimicrobials than non-biofilm producing organisms[60].

AH Plus is an improved epoxy resin-based sealer. AH Plus has retained the epoxy resin "glue" of AH 26 and is also free of formaldehyde release. The antimicrobial effect of epoxy resin-based sealers might be related to the release of formaldehyde during the polymerization process[37].

RealSeal self-etch sealer is a dual cure hydrophilic methacrylate resin based endodontic sealer. In the oral environment, these hydrophilic resins can absorb water and release free unreacted monomers[46]. These unreacted monomers might be the reason for antimicrobial activity of RealSeal self-etch sealer.

iRoot SP is a new endodontic sealer, chemically based on bioaggregate, a ceramic root-canal filling material[65]. The sealer is a complex form of calcium phosphate calcium silicate cement and calcium oxide. Hydration reaction of Calcium silicate produces calcium silicate hydrogel and calcium hydroxide with the help of moisture from dentin[50]. Calcium hydroxide partially reacts with the phosphate to form hydroxyapatite and water[63]. This water is supposed to initiate the reaction cycle again and reacts with un-reacted calcium silicates to produce calcium silicate hydrogel and calcium hydroxide.

The manufacturer suggests the setting time of sealer is 4 hours. The pH of sealer is higher than 12 during the setting process, which increases its bactericidal properties.

The hydration reaction of calcium silicate is as follows:

$2[3CaO.SiO2] + 6H2O \longrightarrow 3CaO.2SiO2.3H2O + 3Ca(OH)2$

$2[2CaO.SiO2] + 4H2O \longrightarrow 3CaO.2SiO2.3H2O + Ca(OH)2.$

The precipitation reaction of calcium phosphate apatite is the following reaction:

$7Ca(OH)2 + 3Ca(H2PO4)2 \longrightarrow Ca10(PO4)6(OH)2 + 12H2O.$

The antimicrobial effect of iRoot SP sealer is suggested as a combined effect of hydrophilicity, high pH and active calcium hydroxide diffusion.[28]

In the present study, the comparison between different groups for antibacterial activity against E. faecalis showed superior antibacterial activity of Group I (AH Plus) as compared to Group II (RealSeal self-etch) and Group III (iRoot SP) during each time period of study except fresh samples. The reason for this deviation from previous studies might be attributed to the slow setting (8 hours) and slow release of formaldehyde from AH Plus[20]. After setting, the antimicrobial activity of AH Plus increased consistently throughout the study, which explain the mixed results regarding the antimicrobial activity of AH Plus from previous studies.

RealSeal self-etch sealer showed no much difference in antimicrobial activity throughout the study, this is in accordance with the study done by **Hui Zhang et al (2009)**[28] who found similar results with Epiphany self-etch (composition similar to RealSeal self-etch)

The outcome of present study had shown that there is no statistically significant difference in antibacterial activity between Group II (RealSeal self-etch) and Group III (iRoot SP) except at 24 hours aged samples.

iRoot SP showed maximum antimicrobial activity on 7 days and minimum on 1 day samples. This might be due to less release of calcium ions after 24 hours (0.204 mg/L) and maximum release on 7th day (1.108mg/L). This difference in calcium ion release might be associated with the final setting time of this material that occurs between 7 and10 days in the moist environment as suggested by **Loushine BA et al (2011)** [38].

DENTINAL TUBULES PENETRATION DEPTH

The primary etiological agents of peripical periodontitis, such as facultative and anaerobic microbial species have the propensity to penetrate deep into the dentinal tubules. Bacterial penetration into the dentinal tubules may reach 100- 1,000 µm and even more in the absence of smear layer[24]. Therefore, the penetration of root canal sealer into dentinal tubules may be beneficial, because various studies have shown the antimicrobial property of various root canal sealers.

Even if the bacteria that may remain in the dentinal tubules were not killed, the sealer will act as a blocking agent that may prevent further bacterial mitotic activity or inactivate them[52].

Scanning Electron Microscope (SEM) has been used in various studies to evaluate the penetration of root canal sealers into dentinal tubules[2,34]. Scanning Electron Microscope provides a number of advantages. This technique allows observation of dentinal tubules and accurate measurement of penetration depth of sealer into dentinal

tubules at a higher magnification such as at 1000x, 3000x or 5000x. The adaptation of sealer to the tubules can be seen in detail at high magnification.

Another major advantage is that, it allows for the observation of root canal sealer within the tubules at distant sites from the canal wall where the density of tubules is less e.g. apical area of root canal. Therefore, in the present study Scanning Electron Microscope (SEM) has been chosen as the tool to measure the dentinal tubule penetration depth of root canal sealers.

However, the disadvantages of Scanning Electron Microscope include its inability to obtain a detailed overall view at low magnification and potential for producing artifacts during the preparation of samples. All these features make systematic analysis more difficult for analysis[34].

Root canal anatomy is the most complex anatomy, having fins, isthmi, accessory canals, lateral canals and curves in mesiodistal and buccolingual directions. To avoid influence of all these anatomical variations maxillary central incisors with single canals have been used in this study. To standardize the length of canal teeth were decoronated and canal length kept constant for all the samples up to14 mm.

Nickel titanium rotary instruments are providing a number of advantages in canal preparation as compared to manual technique which includes its speed, ease and convenience. A study by **Nakamura VC et al (2012)**[42] had reported no statistically significant difference between canal preparation techniques by using manual K type instrument till size 50 or ProTaper Universal instrument till F_5 on microbes and smear layer removal. Taking this factor into account ProTaper Universal rotary instrumentation till size F_5 has been selected for canal preparation in the present study.

Instrumentation during root canal therapy produces an amorphous, irregular and granular layer covering dentin, known as smear layer. This consists of inorganic debris and organic components, such as pulp tissue remnants, saliva, odontoblastic processes, bacteria and blood cells. The smear layer plays an important role in root canal therapy because it affects the adaptation of filling materials to the root canal walls. Application of EDTA and NaOCl removed the smear layer completely and allowed sealer to penetrate into the dentinal tubules.

There will be more demineralization effect of EDTA on dentin, if it remains in contact with dentin for longer duration. Therefore, a contact period of 1 min duration between EDTA and dentinal wall is considered sufficient to avoid the destructive effect of EDTA on dentinal walls as according to the study by **Moon YM et al (2010)**[41]**.**

According to manufacturer's instruction NaOCl cannot be used as a final irrigant with RealSeal self-etch sealer because it will affect the physical properties such as setting of the sealer. Therefore, distilled water is used as a final irrigant to remove any chemical from the canal and avoid unnecessary chemical interactions between root canal sealers and irrigants.

Various factors can influence the capacity of dentinal tubule penetration of endodontic sealer such as: the surface activity of the sealer, the contact angle between sealer and the dentinal walls, the diameter of the opened dentinal tubules and the employed obturation technique[23]. Lateral compaction of gutta-percha with root canal sealer is the most widely used method to obturate the root canals. A study by **Gustavo De-Deus et al (2004)**[23] had suggested no significant difference in penetration depth of root canal sealers into dentinal tubules by using either lateral compaction technique or

single cone technique for obturating root canals. In the present study single cone technique is preferred as a method of obturation because of the ease of this technique and single cone will snugly fit into the root canal.

There are various methods for placement of sealer in the root canal which includes paper points, file, a Lentulo spiral, a root canal spreader, ultrasonics and master gutta-percha cone. Studies have suggested that distribution of sealer in canal walls is not affected by the method of placement[26, 32, 35, 62].

Both horizontal and longitudinal sectioning method can be employed to measure the dentinal penetration tubule depth of root canal sealers. Both the techniques have their own advantages and disadvantages. Disadvantage of horizontal sectioning is that only a small area of canal can be observed and analyzed. This might be the reason of great disparities in maximum penetration depth in various studies. The disadvantage of longitudinal sectioning is that there is possibility of missing areas of deeper penetration depth, because circumferential dentin surrounding the canal cannot be completely observed and evaluated[55]. However, different sectioning methods gave different measurement depth in tubules depending upon the technique employed.

Depth of root canal sealers has been evaluated in three different areas such as coronal, middle, and apical third of root canal. This is because of difference in tubule density, tubule diameter and tubule number in different areas of root canal.

In the present study, all the three groups had shown maximum penetration depth at coronal third followed by the middle third, and least in the apical third. This outcome is speculated in agreement with **Rupali Chadha et al (2012)**[53] who reported similar results and stated that this difference in penetration could be due to the presence of significantly

higher density of dentinal tubules with greater diameter at the coronal and middle third, as compared to the apical third.

The results of present study has shown maximum mean penetration depth of Group I (AH Plus) is 64.89µm which is in accordance with the study by **Kokkas et al (2004)**[2] who reported mean maximum penetration depth of 54.6µm by using longitudinal sections under SEM.

There is no statistically significant difference in penetration depth between Group I (AH Plus) and Group II (RealSeal self-etch). This finding is in accordance with the study by **Noushin Shokouhinejad et al (2011)**[58], compared AH Plus, Epiphany and Epiphany self-etch (composition similar to RealSeal self-etch) and found maximum penetration depth 22.07µm and 21.50µm for AH Plus and Epiphany self-etch respectively.

The mean maximum penetration depth of Group III (iRoot SP) is 108.57µm. There is no previous study regarding the dentinal tubule penetration depth of this sealer. Group III (iRoot SP) has maximum penetration depth into the dentinal tubules as compared to Group I (AH Plus) and Group II (RealSeal self-etch).

The penetration of root canal sealers into the dentinal tubules might be influenced by variations in the physicochemical properties of the sealers[45].

To penetrate completely into dentinal tubules the material should make a good contact with the tooth structure or completely wet the tooth structure which in turn depends upon the contact angle. "Contact angle is the angle of intersection between a liquid and a surface of a solid that is measured from the solid surface through the liquid

to the liquid/vapor tangent line originating at the terminus of the liquid/solid interface. No wetting occurs at a contact angle of 180^0 and complete wetting occurs at an angle of $0^{0"}$.

All the root canal sealers used in this study were in paste form hence, more the sealer will wet the tooth or make less contact angle, more will be the penetrability of sealer into dentinal tubules.

As from study by **Hui Zhang et al (2009)**[28] the contact angle of iRoot SP sealer is 25^0 on fresh samples and as the number of days increased contact angle decreased to less than 5^0 on 7 days samples. On the other hand contact angle of AH Plus was 66^0 on fresh day samples and it increased to 83^0 on 7 days samples. Epiphany self-etch sealer having similar composition to RealSeal self-etch sealer showed contact angle 50^0 on fresh day samples and 35^0 on 7 days samples.

In the present study before analyzing the samples under SEM, samples were kept for 7 days under relative humidity to allow complete penetration of sealers into the dentinal tubules. As from above discussion, it is established that after 7 days iRoot SP root canal sealer has lowest contact angle as compared to the other two sealers. Therefore, iRoot SP root canal sealer has more chances of wetting the canal surface completely and penetrates deep into dentinal tubules.

Another factor which contributes towards the more penetration by iRoot SP sealer is flow. Flow is an important physical property that allows the sealer to fill spaces of difficult access such as isthmus, lateral canals and accessory canals. According to the ISO 6786/2001 recommendations[30], the minimal flow required for root canal sealers is 20 mm.

According to the flow test by **George Tiaccio de Miranda Candeiroe et al (2012)**[18], Endosequence BC having composition similar to iRoot SP Sealer demonstrated flow greater than 20 mm (26.96mm), which is in accordance with ISO 6786/2001 recommendations. However, a high flow might increase the chance of the material extrusion beyond apical foramen. Although it has been demonstrated that the iRoot SP Sealer presents low cytotoxicity[38, 66,67], care should be taken to avoid overfilling.

Another possible explanation for greater penetration depth into dentinal tubules by Group III (iRoot SP) is its small particle size, which is less than 2µm as suggested by the manufacturer. The diameter of dentinal tubules varies from 1µm to 2.5µm and traverses the entire thickness of dentin from the CDJ or DEJ to the pulp. Therefore, due to narrow particle size than the dentinal tubules diameter iRoot SP root canal sealer may have more chance to penetrate to a greater depth into dentinal tubules.

Up to now, various properties such as biocompatibility, pH, antibacterial effect, calcium release, flow, setting reaction, dislocation resistance and radiopacity has been evaluated for iRoot SP root canal sealer. All these investigations and present study established that iRoot SP sealer is superior as compared to existing root canal sealers and provides an adequate outcome towards its use in clinical situations.

Summary

This study investigated the antimicrobial activity and dentinal tubule penetration depth of AH Plus, RealSeal self-etch and iRoot SP root canal sealers.

The test groups considered were

Group I - AH Plus, an epoxy resin based root canal sealer

Group II - RealSeal self-etch, a resin based root canal sealer

Group III - iRoot SP, a bioceramic based root canal sealer

Direct contact test was used to study the antibacterial property of tested materials. **Enterococcus faecalis** was used as the test organism. 96 well microtiter plates were used for analyzing each Group for **7 days, 3 days, 24 hours after set and freshly mixed** (Group A wells). A 10µL bacterial suspension (10^6 bacteria) was placed on the test materials. After ensuring direct contact at 37°C for 1 hour, BHI broth (245µL) was added to each of the Group A wells and gently mixed for 2 min. 15µL was then transferred from Group A wells respectively to an adjacent set of 4 wells containing fresh medium (215µL) designated as Group B. The growth curves from experimental Group A and B were compared with the control bacterial outgrowth A and B respectively for all the study groups. Plates were incubated at 37°C in a humid chamber. Bacterial growth was followed by densitometric measurement in a microplate reader. The OD in each well was recorded every 30 min for 6 hours at 600nm.

For assessment of dentinal tubule penetration depth, 30 freshly extracted single rooted human central incisiors were used. The anatomical crowns were decoronated and canal length standardized to 14 mm. Samples were randomly divided into three groups, each containing 10 samples.

Canal preparation was done with ProTaper Universal rotary system using sodium hypochlorite, EDTA and distilled water as a final irrigant. The three sealers AH Plus, RealSeal self-etch and iRoot SP were coated in prepared and dry canals with the help of Lentulo spiral and obturated with single gutta-percha cone $F_{5.}$

After obturation the samples were kept for 7 days under 100% relative humidity to allow complete setting of sealers. Roots were sectioned longitudinally, examined under Scanning Electron Microscope (450x, 500x, 600x), dentinal tubule penetration depth recorded in µm and results tabulated.

Statistical analysis was performed using one-way ANOVA test followed by multiple comparisons by Tukey's HSD Post-Hoc test.

The results of antimicrobial activity of samples showed greater antimicrobial activity of **Group I (AH Plus)** as compared to **Group II (RealSeal self- etch) and Group III (iRoot SP)** over a period of 7 days time.

There was no statistically significant difference in antimicrobial activity between **Group II (RealSeal self-etch) and Group III (iRoot SP)**.

The results of dentinal tubule penetration depth study showed that **Group III (iRoot SP)** has greater penetration depth (108.57µm) as compared to **Group I (AH Plus)** (64.89µm) and **Group II (Real Seal self-etch) (**63.53µm).

There was no statistically significant difference in penetration depth between **Group I (AH Plus)** and **Group II (RealSeal self-etch)** root canal sealers.

Conclusion

This in vitro study compared the antibacterial activity and dentinal tubule penetration depth of different root canal sealers.

From the present study following conclusions could be drawn:

- ✓ AH Plus performed better in antibacterial activity as compared to RealSeal self-etch and iRoot SP root canal sealer.
- ✓ iRoot SP showed greater depth of penetration into dentinal tubule as compared to AH Plus and RealSeal self-etch.

Within limitations of this study, the new ceramic based root canal sealer iRoot SP has moderate antibacterial activity and greater penetration depth into the dentinal tubules due to its hydrophilic nature. Therefore, its use is beneficial clinically in terms of requirements suggested by Grossman.

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